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Studies on transfer of matter across membranes with special reference to the isolated human amniotic membrane and the exchange of amniotic fluid

BY

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"trac

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r_i

List of symbols

Vectors are denoted by an arrow over the symbol. Species present in "tracer" amounts are denoted by an asterisk as superscript.

A area of membrane.

A_s effective area for solute penetration (p. 27).

A_w effective area for water penetration (defined by equation 29).

D' diffusion coefficient (defined by equation 10).

D "free" diffusion coefficient.

F the Faraday constant.

J flows in irreversible processes.

J_i flow of matter of component i with respect to the centre of gravity (mass per unit time (and area)).

J_i^m flow of matter of component i with respect to laboratory coordinate (mass per unit time (and area)).

L_{ik} phenomenological coefficient relating flows and forces.

Q_i permeability coefficient of component i.

R gas constant.

T absolute temperature.

V_i partial specific volume of component i.

$\tilde{V}_i$ partial molar volume of component i.

X forces in irreversible processes.

X_i force conjugated to the flow J_i.

Z_i permeability coefficient of component i (Q_i per unit area).

a_i activity of component i.

c_i concentration of component i.

d membrane thickness.

e_i partial specific charge of component i.

p pressure.

r_i electrical transport number of component i.

s_i mechanical transport number of component i.

t time.

u_i velocity of component i with respect to laboratory coordinate.

v', v velocity of mass centre with respect to laboratory coordinate.

y_i mass fraction of component i.

μ_i specific chemical potential of component i.

$\bar{\mu}_i$ specific total chemical potential of component i, including electric energy (equation 8).

$\tilde{\mu}_i$ molar total chemical potential of component i.

π apparent osmotic pressure (equation 13).

ϱ_i density (mass per unit volume) of component i.

φ electrical potential.

"For, after all, the origin of the amniotic liquid is in a way a bagatelle."
JOSEPH NEEDHAM in Chemical Embryology.

Introduction

The site and mechanism of exchange of amniotic fluid is far from understood although a number of important physiological and clinical data concerning these problems have accumulated during recent years. It is the object of the present work to give data concerning the part played in this exchange by one of the phase boundaries that separates the amniotic fluid from the maternal compartments, namely the amniotic membrane. The general approach has been to perform experimental studies on the permeability properties of the isolated human amniotic membrane to relevant molecular and ionic species. No data of this nature have been published hitherto.

Furthermore, related to the above stated problem, theoretical and experimental studies concerning some special aspects on matter transfer across membranes are presented. The theoretical basis of the dynamics of matter exchange in the amniotic-maternal system and interpretations of experimental data on these points are treated at some length because it is felt that such aspects have been somewhat neglected.

Chapter I

Theory

1. Some basic concepts and definitions concerning liquid exchange in biological systems

Although a fairly well established terminology exists with regard to the physics of matter transport in free solution and across phase boundaries, this is not the case in more general considerations on transfer of matter in biological systems. Below are given some of the basic concepts and definitions used in the present work.

A (liquid) *compartment* is a phase bounded by *phase boundaries*. From a physiological point of view, the characteristic property of a compartment is, in general, that the state variables of the liquid in it are uniform throughout. To achieve this, the movement of matter across the phase boundaries is supposed to be slow compared to the movement within the compartment.

If the phase is thin compared to the adjacent phases, it is convenient to call it a *membrane*. In general, it should be so thin that for a passive membrane, its production and absorption of energy and matter during approach to the stationary state is negligible compared to the content of energy and matter of the surrounding phases.

The *exchange of a liquid* in a compartment signifies all kinds of matter transfer between the compartment and its surroundings.

A liquid compartment exchanging matter and/or energy with its surroundings is an *open system*. If it contains n components, all conserved and incompressible and at concentrations

c, the continuity equation may be written for the ith component

$$\operatorname{div} \vec{J}_i^m + \frac{\partial c_i}{\partial t} = 0$$

where $\vec{J}_i^m$ is the net flow of i across a unit surface normal to the flux vector. The system as a whole is in a *steady state* if for all components i, $\partial c_i / \partial t = 0$. Accordingly, in a steady state we have also $\operatorname{div} \vec{J}_i^m = 0$. We may, however, require that for all i, $\vec{J}_i^m = 0$, whereby the system is in *equilibrium*. Finally, if for all $i = 1, 2, \ldots k$, $\vec{J}_i^m = 0$ and for all $i = k+1, k+2 \ldots n$, $\vec{J}_i^m$ is a non-zero constant, the system is in *quasi-equilibrium*.

Total flow (*total flux, unidirectional flow*) of a molecular species across a membrane in a specified direction is the rate of movement (amount per unit time and area) of *all* molecules of that species in that direction. In cases when the membrane is thick compared to the dimensions of the migrating species, an equilibrium or steady state condition is necessary for a proper definition. For a non-equilibrium, non-steady state condition it is possible to define total flows only with respect to an arbitrary plane in the (homogeneous) membrane.

Net flow (*net flux*) of a molecular species across a membrane is the algebraic sum of the total flows.

A *passive membrane* is one across which no net flow occurs when the total chemical potential (defined by eq. (8)) of each species is the same on both sides of the membrane.

If the total flows of each species across a passive membrane are equal (zero net flows), the whole exchange of any species across the membrane is caused by *self-diffusion exchange*. Of course, self-diffusion exchange operates also during conditions of finite net flows. The rate of self-diffusion of a component is estimated by measuring the rate of migration of an isotope in tracer amounts of that component with the assumption that the isotopes behave identically in this respect.

If, in our open compartment system, finite net flows of certain species occur in the steady state across the phase boundary shared by the compartment liquid and some morphologically

well defined structures, it is customary to denote such flows as *formation* (*reabsorption*) of the components. There is considerable advantage in such a terminology, but it should be pointed out that net flows of the same magnitude and in opposite direction might well occur simultaneously in macroscopically homogenous structures, perhaps through channels only a few molecular diameters apart. Evidently, in such a situation, the distinction between formation-reabsorption exchange and self-diffusion exchange looses its importance since it escapes experimental measurement.

The *rate of turnover* of a component within a compartment in a stationary state is the fraction of that species that is replaced per unit time by exchange processes with the surroundings. In non-stationary states, turnover rates should be defined with respect to the appropriate time.

The *circulation* of a liquid within a compartment is the (local) convection currents within it.

2. On the theory of irreversible transport of matter through membranes

General remarks. Most of the problems which will be dealt with in the following chapters are concerned with transport of matter through membranes and phenomena related to such transport. In this section, the theory of transport processes in systems relevant to the present work will be briefly outlined.

In biological systems there exist several different "forces" that may give rise to irreversible transport phenomena. Such "forces" are for instance gradients of (or differences in) concentration, temperature and electrical potential. The resulting irreversible phenomena are called flows or fluxes, for example diffusion flows, heat flow and electrical current flow. The thermodynamic theory of irreversible processes (see for instance PRIGOGINE 1949, DE GROOT 1952 and HAASE 1952) is founded on very general considerations of entropy production during the occurrence of irreversible phenomena and on the principle of microscopic reversibility (ONSAGER 1931 a and b). The theory deals with the general laws relating the flows and

the forces provided that linear relations exist between these. The condition of linearity is generally considered to be fulfilled when the deviation from equilibrium of the system is small.

In general a complete description of all driving forces related to matter transport across membranes consists of an evaluation of the gradients within the membrane of all intensive properties of the system and then of finding the boundary conditions for spatial and temporal integration. All driving forces should be expressed in terms of the intensive properties of the phases surrounding the membrane and in theoretical or empirical formulations of the interaction between the membrane molecules and those of the phases. Theories of interaction will depend on the particular model chosen for the description of the membrane, and the choice will determine the resistance offered to the transport of each species present. A complete *thermodynamical* description of the membrane processes may be given without paying any attention to what happens in the membrane itself in the special case in which it can be treated as a surface of discontinuity and when the deviation from equilibrium is small. Thus, STAVERMAN (1952) showed how, in such a situation, a very general treatment of membrane phenomena may be carried out with the help of "irreversible thermodynamics". Apart from the restrictions that follow from the model, this is, in general, not sufficient for the biologist, who, besides wishing to have a more or less complete account of the mechanisms underlying the processes, also wants to predict, at least qualitatively, the events in systems about which he has some ideas. It should be noted however that any specific model proposed and treated with arguments alien to thermodynamics must give results consistent with the pure thermodynamic treatment.

In the most general case, any force may give rise to any flow and accordingly we may write

$$J_i = \sum_{k=1}^{n} L_{ik} \cdot X_k \quad (i = 1, 2, 3, \ldots n) \quad (1)$$

where all the L_{ik}:s with $i \neq k$ determines that part of the flow of i produced by the force X_k, i. e. they are coefficients for interference phenomena such as heat flow caused by concen-

tration gradients or diffusion flow caused by temperature gradients. The relation $L_{ik} = L_{ki}$, due to ONSAGER, is valid when a proper choice is made for the fluxes and forces and is of special interest when a discontinuous system is treated. If a membrane is considered as a phase of finite thickness and its intensive properties depend on the space coordinates, we have a continuous system and may define flows $\vec{J}_i$ by

$$\vec{J}_i = \varrho_i (\vec{u}_i - \vec{v}') \tag{2}$$

where

$$\vec{v}' = \sum_{i=1}^{n} y_i \cdot \vec{u}_i \tag{3}$$

and

$$y_i = \frac{\varrho_i}{\varrho} \tag{4}$$

using

$$\varrho = \sum_{i=1}^{n} \varrho_i \tag{5}$$

The phenomenological relations are

$$\vec{J}_i = \sum_{k=1}^{n} L_{ik} \cdot \vec{X}_k \tag{6}$$

where for isothermal conditions, neglecting gravitational forces,

$$\vec{X}_k = -\operatorname{grad} \bar{\mu}_k \tag{7}$$

Finally, the thermostatical relation below is valid

$$\operatorname{grad} \bar{\mu}_k = V_k \operatorname{grad} p + \sum_{i=1}^{n-1} \frac{\partial \bar{\mu}_k}{\partial y_i} \operatorname{grad} y_i + e_k \operatorname{grad} \varphi \tag{8}$$

In the following, only special cases will be treated, for which the relations will be correspondingly simpler. Especially, the simplification $L_{ik} = 0$ for $i \neq k$ can be used. Furthermore, it will be sufficient to treat transport in one dimension only.

Special considerations. At the present stage of the discussion, the following model is considered (later on, a more detailed model system will be required). A homogeneous membrane of

finite thickness is bounded by two planes normal to the x-axis at $x = 0$ and $x = d$. The phase within the membrane will in general not be homogeneous, i. e. the state variables within the phase will depend on the space coordinates. The state variables will however be assumed to be independent of time, i. e., stationary conditions will be considered. Only transport in the x direction will be treated, and the frame of reference for the transport will be fixed to the membrane. We may define then new fluxes J_i^m by

$$J_i^m = J_i + \varrho_i \cdot v' \tag{9}$$

where the migrating species are allowed to interact during passage. For a binary solution and zero gradient of electrical potential, we may use (6), (7), (8) and (9) to give for component 1 if the pressure gradient is small enough to be neglected in (8)

$$J_1^m = -\varrho D' \cdot \frac{dy_1}{dx} - \varrho_1 \cdot v \tag{10}$$

where $v = -v'$ and

$$D' = (L_{11}/y_2\varrho)(\partial\mu_1/\partial y_1)_{T,p,\varphi} \tag{11}$$

For vanishing net mass flow ($v = 0$), eq. (10) becomes identical with the well known Fick equation for steady state, one-dimensional diffusion. The coefficient of the concentration gradient, D', is usually called the diffusion coefficient.

In the case when the various components pass the membrane without mutual interaction, i. e. molecule by molecule, it is convenient to describe the diffusion with respect to the velocity of the membrane component, which in this case will serve as solvent medium. This description will be almost identical with the barycentric description used above (eq. (2)) in the case when dilute solutions are treated.

In electrolyte solutions, the different diffusion velocities of the various ionic species set up electrical potential gradients. Within membranes, such gradients may conveniently be regarded as a result of two different phenomena, the first one resulting from such differences in diffusion velocities that occur also in free solution and the second one due to differ-

ences imposed by the action of the membrane. The electrical potential difference across the membrane may be written (cf. STAVERMAN 1952, SCATCHARD 1953)

$$\Delta \varphi = - \int_{x=0}^{x=d} \sum_{k=1}^{n} \frac{r_k}{e_k} d\mu_k \qquad (12)$$

where r_k are the local electrical transport numbers, i. e., the relative contribution of the component k to the transport of electricity when there is no gradient of pressure or concentration in the membrane. For special cases, eq. (12) reduces to well known relations for membrane or liquid junction potentials (cf. NERNST 1888 and 1889, PLANCK 1890 a and b, HENDERSON 1907 and 1908, STAVERMAN 1952, TEORELL 1951 and 1953).

Very often the properties of the membrane and its surrounding phases are such that the sum of all J_k^m is different from zero, also in the case that the difference of pressure and electrical potential between the two phases is zero. The resulting mass flow is often called the "osmotic" flow and the hydrostatic pressure difference which can just counteract the flow is accordingly called the "osmotic" pressure. Such a situation arises from the different transport restrictions imposed on the various components by the action of the membrane. If there exist components that cannot pass the membrane at all, the ultimate equilibrium condition for the permeable species will be a situation with a finite pressure difference between the two phases and no net mass flow. If all components can pass the membrane, the ultimate equilibrium situation will be one with equal pressure and composition on both sides of the membrane. However, during approach to equilibrium, transient "osmotic" flows may occur, and if the deviation from equilibrium is maintained, stationary "osmotic" flows will occur. In biological systems such a situation is often present. Despite the steady dissipation of energy by the occurrence of irreversible processes, gradients of chemical potentials of different components across various phase boundaries are constantly upheld by metabolic processes. In analogy with the electrical potential difference within the membrane, we may write for

the "apparent osmotic" pressure difference (cf. STAVERMAN 1952)

$$\Delta \pi = - \int_{x=0}^{x=d} \sum_{k=1}^{n} \frac{s_k}{V_k} d\mu_k \qquad (13)$$

where s_k are the local mechanical transport numbers, i. e. the relative contribution of the component k to the transport of volume when there is no gradient of concentration or electrical potential. During conditions of zero hydrostatic pressure difference between the two surrounding phases, the right hand expression should be looked upon as a driving pressure forcing liquid through the membrane. For a semipermeable membrane this pressure is seen to be identical with the van't Hoff pressure, but for a leaky membrane, the resulting pressure difference will always be less on account of the finite transport numbers of the permeable solutes (cf. STAVERMAN 1951).

The term active transport and its definition have been extensively discussed during the last years (see especially the recent representative Symposium edited by BROWN and DANIELLI 1954), but little is known of the intimate mechanisms of the phenomenon. Generally, active transport across a membrane may be said to occur when the material flows are more or less directly dependant on constant expenditure of energy from metabolic processes in the membrane. The operational definition advocated by USSING (1949, cf. also TEORELL 1949), of which the situation used for the definition of a passive membrane on p. 10 is a special case, seems most important since it is concerned solely with measurable quantities.

3. Further theoretical considerations and a contribution to the theory of ordinary osmosis

Further developments of the general relations obtained in the preceding section should consist of an evaluation of the boundary conditions for integration in terms of external phase composition and of considerations of the mobilities of the different species within the membrane. Such developments

will, besides the use of specific membrane models, require the application of kinetic theories of molecular transport as well as of theories of interaction between the membrane molecules and those of the solution. This puts serious limitations on the treatment. An important step towards the solution is the introduction of the concept of local equilibria at the two boundaries between the membrane and the surrounding phases. If the rate processes within the membrane are slow compared to those at the boundaries, such a concept may be justified. In the stationary regime, then, the distributions of molecular species between the membrane and the external phases are practically equilibrium distributions upon which thermostatic relations can be applied.

From time to time various model theories have been proposed to account for different membrane phenomena. Recently, a simple theory has been advanced (PAPPENHEIMER, RENKIN and BORRERO 1951, PAPPENHEIMER 1953) to describe restrictions in mobility that occur within the membrane for purely steric reasons. Experiments on passage of uncharged molecules across porous membranes (RENKIN 1954) are in fairly good agreement with this theory, which gives the mobility in the membrane, D'/RT, as a function of the "free" mobility, the dimensions of the (spherical) molecule and the dimensions of the membrane network for the special case that this can be described by circular channels perpendicular to the membrane surface.

Relatively advanced theories have been formulated and used for electro-chemical and electro-kinetic phenomena in membranes (TEORELL 1935, 1936, 1951 and 1953, MEYER and SIEVERS 1936, SCHLÖGL 1955; these papers also contain discussions on earlier works). The original TEORELL–MEYER–SIEVERS theory attempted a generalisation of earlier treatments, calling attention to the fact that certain complicated ion transport phenomena across membranes could be dealt with by the application of simple thermostatic relations on the local equilibria at the boundaries of the given membrane model. Their model permits the membrane matrix to contain fixed charges, the mobility ratio of the mobile ions in the membrane may or may

not be the same as that in free solution. Together with the PLANCK (1930) treatment on the mixture within the membrane, the extended theory (TEORELL 1951 and 1953) covered a comparatively large field of transport phenomena. However, processes related to net mass flow such as ordinary and anomalous osmosis and other electro-kinetic phenomena were not included in the treatment. The mass flow and its influence on the other processes were discussed by the last mentioned author in terms of the linear velocity of the water flow. This is essentially also the method used by USSING (1952, KOEFOED-JOHNSEN and USSING, 1953).

SCHLÖGL (1955) pointed out that the TEORELL–MEYER-SIEVERS concept of a Donnan equilibrium distribution at the boundaries demands a finite pressure gradient within the membrane (even in the absence of a pressure difference between the two external phases). His extension of the theory made possible a quantitative treatment of the phenomenon of anomalous osmosis for which no satisfactory theory was available.

The phenomenon of ordinary osmosis has been known for more than 200 years and its importance in biology has been recognized almost as long. Little attention has been devoted to describing its mechanism in terms of current concepts of membrane phenomena (see however LAIDLER and SHULER 1949). Stated from a thermodynamic standpoint, the solvent merely moves from a region where its free energy is large to a region where its free energy is small. Very often, the solvent is said to *diffuse* through the membrane because of the difference in thermodynamic properties between the two phases, and diffusion equations (such as (6)–(8) have been applied to describe "osmotic" water flow (GOLDMANN 1951, CHINARD 1952, HARRIS 1956). In view of recent concepts and definitions of the term diffusion (ONSAGER 1945, DARKEN 1948, HARTLEY and CRANK 1949, PRAGER 1953, see also DE GROOT 1952) such a statement not only seems inconvenient but may lead to quite erroneous results (GARBY 1955 a, USSING 1952 and USSING and ANDERSEN 1955).

An attempt to form a contribution to the theory of ordinary

osmosis is given below. The occurrence of a pressure gradient within the membrane (present also in the absence of any pressure difference between the two surrounding phases) is emphasized. The pressure gradient arises as a direct result of the interaction between the membrane molecules and the molecules of the solution and is the immediate cause of the (hydrodynamic) "osmotic" flow. The pressure drop may be given in quantities amenable to experimental measurements. In its basic concepts, the treatment leans heavily on SCHLÖGL's extension of the TEORELL–MEYER–SIEVERS theory.

The model which will be considered is the following: Two liquid phases, I and II, each containing the same solute and solvent species, are separated by a membrane of a thickness that is large compared to the dimensions of the molecules of the solution. The phases I and II will, in general, differ in pressure and composition, but isothermal conditions will be assumed. Electrical phenomena will also be excluded. The membrane network is homogeneously spaced and of such structure that the solvent is able to form a continuous phase throughout the membrane. The channels formed by the solvent may or may not, for instance on account of steric reasons, permit the passage of solute molecules. There is finite resistance to the passage of solvent and solute molecules through the membrane, but it is assumed that the transport of molecules in the phases I and II is rapid enough to secure homogeneous mixing, a situation that may be obtained by mechanical stirring. The system may be divided into three different subsystems. The phase boundaries, between I and the membrane and between II and the membrane, together with the phases immediately adjacent to these, should be looked upon as discontinuous systems, i. e., changes in intensive properties are assumed to take place almost discontinuously at the phase boundary. The third subsystem, the membrane phase *m*, is a continuous system, i. e., the intensive properties depend on the space coordinates. In a stationary state, a constant distribution of the intensive properties between the different phases I, II and *m* is attained. With the assumptions stated above, the distribution at the two phase boundaries is practically an

equilibrium one. In the particular model studied by TEORELL-MEYER–SIEVERS, a Donnan equilibrium distribution was assumed. However, the situation may be generalized to include also distributions that occur from constraints other than electrical, for instance "chemical" constraints due to differential "solubility". In fact, as TEORELL (1953 p. 363) points out: "There is, however, nothing to prevent the choice of any other theoretical or empirical relation between the bulk and membrane interface concentration of the mobile ions". In the present system, we may therefore write for the solvent

$$\tilde{\mu}_w^I = \tilde{\mu}_w^{mI} \quad \text{and} \quad \tilde{\mu}_w^{II} = \tilde{\mu}_w^{mII} \tag{14 a, b}$$

where the subscript refers to the solvent and the superscripts to the different phases described above.
Furthermore

$$\tilde{V}_w^I p^I + RTln\, a_w^I = \tilde{V}_w^{mI} p^{mI} + RTln\, a_w^{mI} \tag{15 a}$$

$$\tilde{V}_w^{II} p^{II} + RTln\, a_w^{II} = \tilde{V}_w^{mII} p^{mII} + RTln\, a_w^{mII} \tag{15 b}$$

which for $\tilde{V}_w^I = \tilde{V}_w^{II}$; $\tilde{V}_w^{mI} = \tilde{V}_w^{mII} = \tilde{V}_w$ and $p^I = p^{II}$ gives

$$p^{mI} - p^{mII} = \frac{RT}{\tilde{V}_w} ln \frac{a_w^I}{a_w^{mI}} - \frac{RT}{\tilde{V}_w} ln \frac{a_w^{II}}{a_w^{mII}} \tag{16}$$

or

$$\Delta \pi = \frac{RT}{\tilde{V}_w} ln \frac{a_w^I}{a_w^{II}} - \frac{RT}{\tilde{V}_w} ln \frac{a_w^{mI}}{a_w^{mII}} \tag{17}$$

For dilute solutions this may be written

$$\Delta \pi = RT \Delta c_s^b - RT \Delta c_s^m \tag{18}$$

where the subscript refers to the solute in a binary system and the superscripts *b* and *m* denote the bulk and membrane phases. The result so far shows that the pressure drop within the membrane, the driving pressure, can be given in terms of bulk and membrane concentrations of the solute. The relation between these concentrations should reflect the differential action of the membrane on the solvent and solute respectively and may be further evaluated as follows.

The flow of tagged solute molecules across a membrane when the total solute concentration is the same on both sides of the membrane, is

$$J_{s^*}^m = Q_{s^*} \cdot \Delta c_{s^*}^b = D_{s^*} \cdot (A/d)_s \cdot \Delta c_{s^*}^b \tag{19}$$

or

$$J_{s^*}^m = D_{s^*} \cdot (A/d)_w \cdot \Delta c_{s^*}^m \tag{20}$$

which define the quantities Q_s, $(A/d)_s$ and $(A/d)_w$ and give

$$\Delta c_{s^*}^m = Q_{s^*} \cdot \Delta c_{s^*}^b / D_{s^*} (A/d)_w \tag{21}$$

The corresponding tracer permeability coefficient Q_w may be given as

$$Q_{w^*} = D_{w^*} (A/d)_w \tag{22}$$

Inserting (22) in (21) and using this in (18), we obtain

$$\Delta \pi = RT \cdot \Delta c_s^b \left(1 - \frac{Q_{s^*} D_{w^*}}{Q_{w^*} D_{s^*}}\right) \tag{23}$$

Our first conclusion is that eq. (23) holds true for the two extreme conditions: the perfectly semipermeable membrane and the membrane that shows no differential restriction to the species involved. In the first case, Q_{s^*} is zero and $\Delta\pi$ becomes identical with the van't Hoff relation. In the second case, the ratio $Q_{s^*}D_{w^*}/Q_{w^*}D_{s^*}$ is equal to unity and the apparent osmotic pressure becomes zero. This is the case of pure interdiffusion.

LAIDLER and SHULER (1949 a and b), performing an essentially kinetic treatment of ordinary osmosis, arrived at an expression similar to (23) for the apparent osmotic pressure. Their treatment is, however, valid only in the case when the various species pass the membrane without mutual interaction and is thus not general.

Eq. (23) provides an expression for the driving force for liquid flow in homoporous membranes due to a difference in solute concentration on both sides of the membrane. When the hydrostatic pressure difference between the two phases is finite, this may be added directly to the $\Delta\pi$ to give the total driving force for flow, just as is done in osmometry with semipermeable membranes. Such a summation has however been

made also in the case of heteroporous membranes (PAPPENHEIMER, RENKIN and BORRERO 1951, PAPPENHEIMER 1953), a procedure which may lead to great errors. The expression for the flow of liquid across a heteroporous membrane in terms of hydrostatic pressure difference, solute concentration difference and relative restriction of solute-solvent is

$$B = \Delta p \sum_{i=1}^{n} k_i + \sum_{i=1}^{n} k_i \Delta \pi_i \qquad (24)$$

where B is the flow rate, Δp is the difference in hydrostatic pressure between the two surrounding phases, k_i is a coefficient expressing the conductance to flow in the ith pore class. The summation is over all pore classes and $\Delta \pi_i$ is given by eq. (23) as applied to the appropriate pore class.

Chapter II

Experiments on matter transfer across membranes with simultaneously occurring bulk flow

In the following chapter, experiments will be presented that deal with some simple phenomena related to transfer of matter through membranes across which net mass flow occurs. The purpose has been to obtain experimental evidence as to the applicability of eq. (10) and relations derived from it on some relatively well defined membrane systems. Preliminary reports on such attempts have been given elsewhere (GARBY 1954 a and b).

1. The kinetics of transfer of sucrose against bulk flow in some porous membranes

Eq. (9) describes the relative rates of transport of any component along a laboratory coordinate due to diffusion and convection. MANEGOLD and SOLF (1932), employing the relation given by HERTZ (1922, 1923), showed how the integration for a simple case, such as that described by eq. (10) with the conditions $x = 0$; $y_1 = y_1$ and $x = d$; $y_1 = 0$, gives

$$J_1^m = \frac{y_1 \varrho \cdot v}{exp\,(v \cdot d/D'_1) - 1} \tag{25}$$

where v is the rate of flow of solution across the membrane divided by A_w and where $A_w = A_s$.

This may be written

$$\frac{Q_1}{A_s} = \frac{v}{exp\,(v \cdot d/D_1) - 1} \tag{26}$$

where Q_1 is a permeability coefficient which characterizes the permeation across the membrane under unit concentration difference and given mass flow and where D'_1 has been replaced by D_1.

Methods. The experimental arrangement is shown in figure 1. A Perspex diffusion chamber contained two compartments separated by a membrane. One compartment, kept at a constant pressure which could be varied at will, was connected to a large reservoir containing distilled water. The fluid content of this compartment was constantly renewed from the reservoir so as to keep the concentration of any species except water practically at zero. The other compartment was open and filled with sucrose solution. Stirring in both compartments

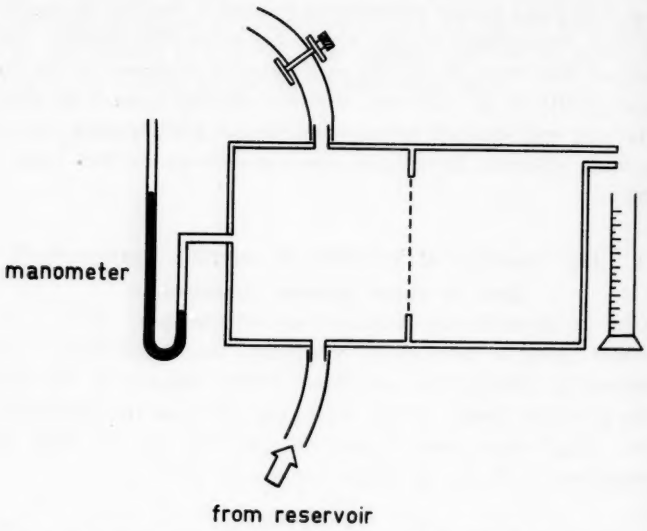

Fig. 1. The two-compartment diffusion chamber with the compartment under pressure to the left and the open compartment to the right. The interrupted line denotes the membrane.

was accomplished by the arrangement shown in figure 2. Two rotating horse-shoe magnets were placed close to the ends of the two cylindrical compartments. In the inside of each compartment a plastic covered iron bar, upon which thin Perspex wings were attached, followed the rotating magnetic field around an axis fixed in the centre of each compartment. The volume of each compartment was about 20 ml. After filling

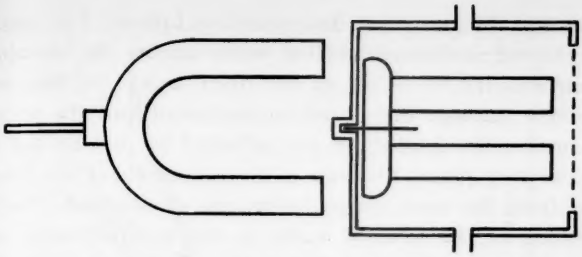

Fig. 2. The stirring device in the left compartment, the horse shoe magnet and the iron bar with its attached plastic wings. The same arrangement was used in the right chamber.

both chambers, the pressure difference was adjusted so that a certain net mass flow across the membrane was obtained. The concentration of sucrose in the solution escaping from the small outlet of the open compartment was measured at certain time intervals, usually every 10th minute. The magnitude of the net mass flow was also recorded. The concentration of sucrose in the open chamber varied between 0 and 40 mM/l. It was estimated by density measurements, the density gradient tube of LINDERSTRÖM-LANG (1937) being used.

The rate of disappearance of sucrose out of the open chamber may be given by

$$\frac{dn}{dt} = -Q_1 c_1 - k_2 c_1 \tag{27}$$

where n is the amount of sucrose in the compartment and k_2 is the rate of flow (volume per unit time) of solution out of the compartment. Integration between $t = 0$ and $t = t$ gives

$$Q_1 = \frac{G}{t} ln \frac{c_1^0}{c_1^t} - k_2 \tag{28}$$

where t is the time interval, G is the volume of the compartment.

The procedure permits measurements of Q_1 for any value of k_2. The coefficient k_2 is equal to $v \cdot A_w$. It remains to show that $A_w = A_s$ and to estimate this area.

It may be assumed that the effective area for water flow is the same as the effective area for water diffusion as measured

by tracers. This area was determined as follows. The permeation rate of deuterium-labelled water across the membrane was determined in the set-up described above. Distilled water circulated through the closed compartment and the pressure drop across the membrane was adjusted so that no net flow of water took place. The rate of disappearance of the labelled water from the open compartment was determined. The concentration of the labelled water in this compartment varied between 6 and 1 % (*w*/*w*) during the experiment. Control experiments showed that the rate of disappearance was completely determined by migration across the membrane, exchange of deuterium and deuterium oxide with water in the air or with the Perspex material being negligible. This was done by measuring the change in concentration of a deuterium-oxide solution in water kept in a Perspex vessel of the same dimensions as the compartment used above. The concentration of the labelled water was determined in the density gradient tube according to LINDERSTRÖM-LANG, JACOBSEN and JOHANSEN (1938).

The effective area for water diffusion may then be evaluated by

$$A_w = \frac{d \cdot G}{D_w \cdot \Delta t} ln \frac{c^0}{c^t} \qquad (29)$$

where G is the volume of the compartment, t is the time interval and c is the concentration of deuterium oxide. The temperature was 18° C and $D_w = 1.95 \times 10^{-5}$ cm sec^{-1}.

The value of D_w and the use of deuterium oxide as a label for H_2O requires some comments. The self-diffusion of water has been measured by a number of investigators using different methods, different labels and different label concentrations (ORR and BUTLER 1935, TEMKIN 1935, LAMM 1937, RÖGENER-LEIPZIG 1941, WANG 1951, GRAUPNER and WINTER 1952, PARTINGTON, HUDSON and BAGNALL 1952, CUDDEBACK, KOELLER and DRICKAMER 1953, WANG, ROBINSON and EDELMAN 1953 and LONGSWORTH 1954). There is a considerable disagreement among the various estimates and it is difficult to evaluate the true value from all data as little is known of the causes of discrepancy (cf. MILLS and ADAMSON 1955 and MILLS 1955). Values of 1.95×10^{-5} cm sec^{-1} at 18° C and 3.03×10^{-5} cm sec^{-1} at 37° C for deuterium oxide are probably correct within 5 % and are in close agreement with those obtained by LONGSWORTH using a Rayleigh interferometric technique in a modified Tiselius diffusion cell. Tritiated water gives about the same values (WANG et al. 1953),

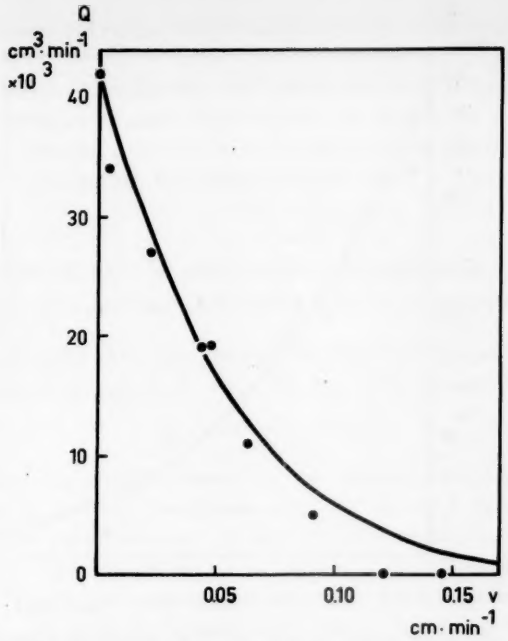

Fig. 3. The permeability coefficient Q for sucrose permeation across "Membranfilter mittel" plotted against the linear velocity v of the flow of solution across the membrane. The curve corresponds to eq. (26).

but widely different values are reported for water tagged with O^{18}. There are no reasons to believe that the self-diffusion of H_2O differs appreciably from the value chosen here.

The effective area for sucrose diffusion was evaluated as follows. In the same set-up as that used for determination of the rate of labelled water diffusion, the rate of sucrose diffusion across the membrane was determined. For that experiment, eq. (29) is also valid when A_w is substituted by A_s and D_w by D_s, the free diffusion coefficient of sucrose under the appropriate conditions. At 18° C, the latter is 0.42×10^{-5} cm sec^{-1} (GOSTING and MORRIS 1949).

Results. Experiments were made on two different membranes: "Membranfilter mittel" (Membranaktiengesellschaft,

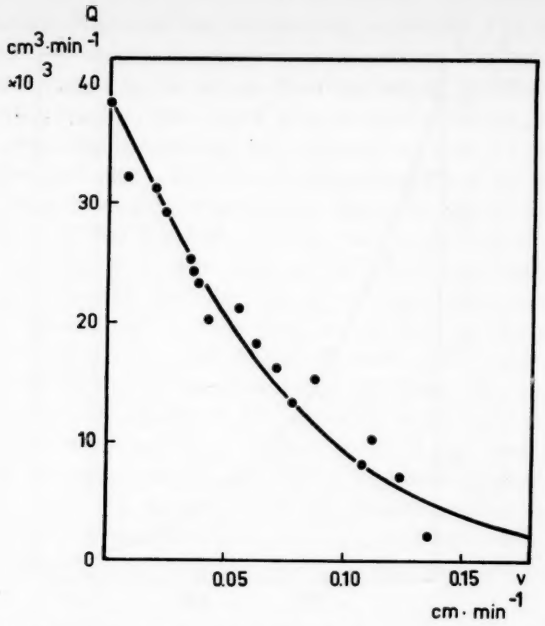

Fig. 4. The permeability coefficient Q for sucrose permeation across "Membranfilter fein" plotted against the linear velocity v of the flow of solution across the membrane. The curve corresponds to eq. (26).

Göttingen, Germany, $d = 0.008$ cm as measured by a micrometer caliper) and "Membranfilter fein" ($d = 0.006$ cm). Determinations of the effective area for water and sucrose diffusion were made only on "Ultrafeinfilter fein" as it turned out that in this membrane, the areas were the same within the experimental error ($A_w/A_s = 1.05$, the values of A_w and A_s corresponding to the periods of sampling were 0.504, 0.483, 0.519, 0.489, 0.511 cm² and 0.472, 0.490, 0.453, 0.483 cm² respectively). It may be safely concluded that the ratio A_w/A_s is even closer to 1 in the more porous membrane and that the membranes do not "differentiate" between the water and the sucrose molecules. In figures 3 and 4, the permeability coefficient Q_1 is plotted against the linear velocity of the mass (water) flow. For each value of k_2, usually 4 or 5 values of Q_1 were obtained. The mean value

of these was taken as representative for the corresponding k_2 values.

Discussion. Considering the errors involved in the method, the experimental values agree reasonably well with the calculated ones. It can therefore be concluded that in the membrane systems investigated, the relation holds true.

2. A special case of the convection-diffusion effect on the concentration difference across a membrane.

Teorell (1951 and 1953) pointed out that during conditions of no net flow of species 1, eq. (10) may be integrated to give

$$y_1^{\mathrm{I}}/y_1^{\mathrm{II}} = exp\,(v \cdot d/D') \qquad (30)$$

where the superscripts denote the two phases surrounding the membrane. Teorell's treatment included charged species and in the case when species 1 in eq. (30) is a single 1 — 1 valent electrolyte, D′ is the diffusion coefficient of the *salt* in the membrane (Teorell, personal communication). Also, Teorell pointed out that although the system will tend to approach the concentration ratio in eq. (30), this will be attained only if "the water content somehow remains constant".

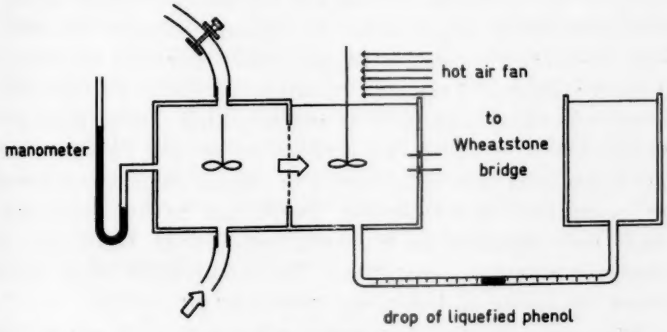

Fig. 5. The two-compartment diffusion chamber with the auxiliary chamber, III, to the right. Between II and III is shown the graduated capillary with its drop of liquefied phenol. The electrodes for conductance measurements and the connections to the measuring bridge are also shown.

Methods. The experimental set-up is shown in figure 5. The unit consisted of the two Perspex cells I and II. A membrane separated the two compartments I and II, both containing a KCl solution. Compartment I and its auxiliaries were the same as those used in the experiments described previously (cf. figure 1 and 2). The other cell, containing compartment II was stirred by a rotating propeller and was open and connected at its bottom to another chamber, III, by means of a graduated capillary tube. In this tube a small drop of liquefied phenol was introduced. Any small volume change in compartment II was transmitted to the capillary tube and forced the drop to move. In order to keep the volume in the compartment II constant, i. e. constant position of the drop, when there was a mass flow from the other compartment, warm air from an electrically heated fan, especially adapted for the purpose, was blown parallel to the open surface of the compartment. By varying the temperature and the flow rate of this air stream, it was possible to balance the volume increment due to flow from the other chamber. In chamber II were also mounted electrodes of platinized platinum for conductance measurements of the KCl solution in this compartment. The conductance was obtained by means of a Wheatstone bridge arrangement fed with 1000 c. p. s. from a sine wave generator. The detector was a Philips cathode ray oscilloscope. Sharp tuning was obtained by adjustments of a small condensor in series with the known resistance. During actual operation, resistance could be measured with an accuracy of about 1: 1000. The temperature in compartment II was measured with an accuracy of about 0°.01 C and varied between 22°.65 and 22°.75 during the experiment. The temperature in compartment I, measured at its outflow, varied between 23°.30 and 23°.70. Concentrations were obtained from conductance values by means of standard solutions; corrections for temperature were made using the values of JONES and PRENDERGAST (1937).

The measurements were carried out as follows. A certain rate of mass flow through the membrane was accomplished by adjusting the pressure difference between the two compartments to a certain value. The temperature and rate of flow

of the air from the fan were adjusted so that no volume changes occurred in compartment II, i. e., no movement of the drop in the capillary tube. The concentration of the KCl solution in compartment I was 10 mM/l and kept constant by vigorous circulation. The concentration of the KCl solution in compartment II that remained constant for at least 10 minutes was recorded. The ratio of this concentration to that in compartment I is an estimate of the left hand side in eq. (30).

The linear velocity of the mass flow in the membrane during the above conditions was estimated as follows. The rate of volume flow was determined by replacing compartment II by another half cell that permitted the excess liquid to drip into a measuring cylinder. The linear rate of flow was then determined in the same way as described in the previous section (cf. eq. (29)). An estimate of the quotient A_w/d was obtained. Dividing the flow rate by this quotient, an estimate of the product $v \cdot d$ was obtained.

The assumption in eq. (30) that the bulk and membrane-bulk interface concentrations are the same may be justified in this case from the fact that in the system ("Ultrafeinfilter grob", d = 0.015 cm, Membranaktiengesellschaft, Göttingen, Germany), the "osmotic" flow for a concentration difference of KCl of 10 mM/l between the two phases was experimentally determined to be less than 3 % of the flow calculated by the van't Hoff pressure and the flow resistance of the membrane. Such a result is consistent only with very nearly identical bulk and interface concentrations. This finding has also a bearing upon the replacement of the diffusion coefficient D' for KCl in the membrane by the "free" diffusion coefficient D, a replacement which is required for the calculations that follow. Its justification can also be seen from the following considerations. The Poiseuille law may be used as follows to give an estimate of the pore dimensions in the membrane

$$B = \frac{A_w \cdot r^2 \cdot \Delta p}{8 \cdot \eta \cdot d} \tag{31}$$

where B is the volume flow rate across the membrane, r is the radius of the channels piercing the membrane perpendicularly

to the membrane surfaces, η is the viscosity of the solution and A_w is the effective area of the membrane for flow. In this case, A_w was estimated from the water content of the membrane. The pore radius thus calculated was about 350 Å. It should be pointed out that this estimate is probably a minimum one since the length of the channels tends to be underestimated by a factor of about 3 in eq. (31) (BJERRUM and MANEGOLD 1927, cf. also RENKIN 1954). If the value of A_w/d as estimated from the diffusion of labelled water across the membrane is used in eq. (31), the radius becomes 430 Å. Though admittedly rough, the calculations indicate that negligible error is made when D' is replaced by D for KCl in the membrane.

Results. When a bulk flow of 73×10^{-5} ml sec^{-1} passed the membrane and the volume of the compartment II was constant, the ratio of the experimentally determined concentrations of KCl between the two compartments, y^I/y^{II}, was 1.37.

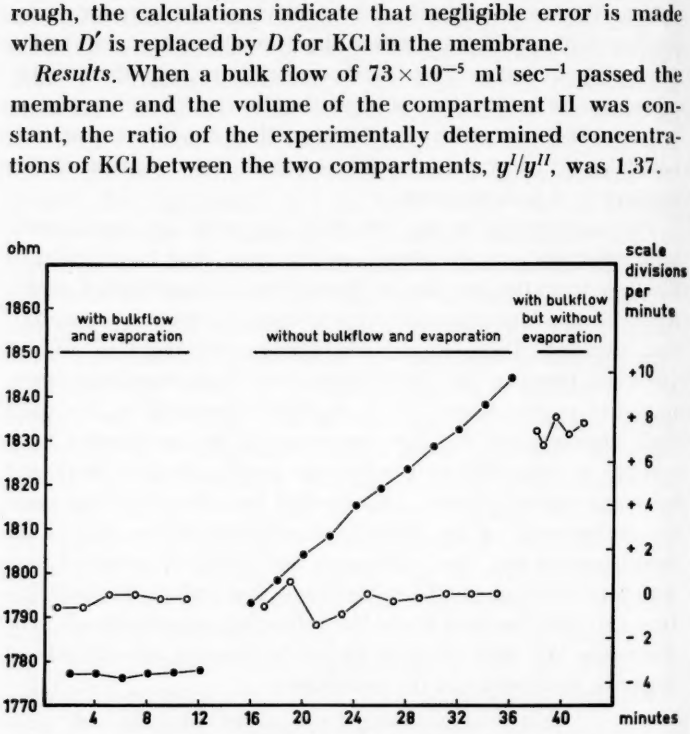

Fig. 6. The response of the apparatus used for the study of the convection diffusion effect (cf. fig. 5). The filled circles denote the electrical resistance of the solution in chamber II. The open circles denote the rate of flow of liquid between chambers II and III, positive numbers denoting flow from II to III.

The ratio A_w/d was 95 cm when a diffusion coefficient of water at 22°.75 C of 2.15×10^{-5} cm sec^{-1} was assumed (cf. p. 26). With a value of 1.82×10^{-5} cm sec^{-1} for D'_{KCl}, (cf. ROBINSON and STOKES 1955, p. 494), the value of $exp(v \cdot d/D')$ was calculated to be 1.52.

Figure 6 shows the variations in the resistance of the solution in compartment II during various conditions. The volume variations are also shown. It is evident that a good balance was obtained.

Discussion. Considering the various experimental errors and assumptions involved, the experimental result agrees satisfactorily with theory. It may be concluded that for the type of membrane used here, the theory describes the process fairly well.

The relation expressed by eq. (30) may be of importance in biological systems (cf. TEORELL 1956). Consider a three-compartment catenary system where the end-compartments I and III are of infinite volumes. There may be bulk flow with regard to a species *i* and water across the boundary between I and II in the direction I to II and "osmotic" flow across the boundary (impermeable to *i*) between II and III in the direction II to III. In the stationary state, compartment II is in quasi-equilibrium with *no net flow of i despite the presence of a chemical or electrochemical potential difference between I and II and finite permittivity of the boundary as measured with the help of tracers.*

Chapter III

The circulation and exchange of amniotic fluid as revealed by earlier investigations

A study of the earlier literature reveals many important contributions regarding the circulation and exchange of the amniotic fluid, in both humans and experimental animals. This literature will be briefly surveyed in the present chapter. NEEDHAM's classical work (NEEDHAM 1931) and a recent monograph by HANON, COQUOIN-CARNOT and PIGNARD (1955) contain a fairly comprehensive review of the earlier work. For convenience, the different approaches to the problem will be dealt with in five sections although such a division necessarily becomes somewhat arbitrary. Some remarks concerning the significance of the earlier findings will be made in this chapter, but a more complete discussion will be postponed until chapter V.

1. The composition of the amniotic fluid

The chemical composition of the amniotic fluid, which at term amounts to about 1000 ml, is similar to that of plasma. In some respects however, there is definite dissimilarity (ZANGEMEISTER and MEISSL 1903, UYENO 1919, GUTHMANN and MAY 1930, MAKEPEACE, FREMONT-SMITH, DAILEY and CARROL 1931, CANTAROW, STUCKERT and DAVIS 1933, URANGA IMAZ and GASCON 1950 and HANON *et al.* 1955). The protein content rarely exceeds 1 % and the total molecular and ionic concentration is lower than that of plasma except for the first 2–3 months of pregnancy. Based on comparisons between the composition of the amniotic fluid and maternal plasma, much

speculation has been made concerning the origin of the former. Many of these considerations were made at a time when there was a paucity of information on the dynamics of matter exchange in biological systems.

The constant protein concentration in the amniotic fluid may well reflect a true equilibrium between this compartment and the maternal and fetal extracellular fluid which is immediately adjacent to the amniotic compartment.

The chloride concentration is very nearly the same as in fetal and maternal plasma (cf. however MAKEPEACE and co-workers 1931) and indicates, because of the Donnan constraint, that the distribution between the amniotic fluid and its immediate side-compartments is a non-equilibrium distribution.

The sodium and potassium concentrations are slightly lower than those of plasma, probably lower than can be accounted for by the Donnan equilibrium.

The considerable difference in total molecular and ionic concentration between the amniotic fluid and the maternal and fetal extracellular fluid is commonly attributed to the addition of hypotonic fetal urine. The osmolarity constantly decreases during pregnancy and at term, the difference corresponds to about 0°.05–0°.07 C in freezing point. Fetal urine is strongly hypotonic compared to the amniotic fluid (MAKEPEACE and co-workers 1931, HANON and co-workers 1955).

The hypothesis that fetal urine enters the amniotic cavity and modifies the composition of the amniotic fluid is furthermore supported by the findings that the concentration of urea, creatinine and uric acid increases during increasing time of pregnancy (GUTHMANN and MAY 1930, FRIEDBERG 1955) and that fetal urine contains these components in higher concentrations than amniotic fluid (MAKEPEACE *et al.* 1931, FRIEDBERG 1955 and HANON *et al.* 1955). At term, considerable concentration differences between the amniotic fluid and maternal plasma exist for these components, the concentrations in the amniotic fluid being higher. Observations by NAESLUND (personal communication) are also interesting in this connection. Besides the findings of significant differences in concentration of uric acid and creatinine between amniotic fluid and

maternal plasma at term, he found that substances giving the xanthoproteic reaction occur in much higher concentrations in the amniotic fluid than in maternal plasma.

The concentration of glucose in the amniotic fluid is considerably lower than in maternal plasma, ranging between some 10 and 70 mg/100 ml with a mean about 30 mg/100 ml. There is some evidence (MAKEPEACE et al. 1931 and ITIZYO 1934) that the concentration is higher during earlier months of pregnancy, a fact that seems to support the view that fetal urine, which contains a very small amount of sugar (MAKEPEACE *et al.* 1931 and HANON *et al.* 1955), contributes to amniotic fluid formation. When the maternal (and fetal) glucose concentration is raised, artificially or in diabetes mellitus, the amniotic fluid concentration also raises, without however reaching the maternal level (OAKLEY and PEEL 1949, PEDERSEN 1954 and MAGNIN, ZAHEDI and PROST 1952).

Concentration gradients across the phase boundaries, of such species as water, glucose, uric acid, creatinine and urea cannot well be assumed to be equilibrium gradients. Therefore, driving forces for transamniotic membrane transport should exist also for these components.

In one special case, steady chemical potential gradients giving rise to no net flow may be established across the phase boundaries despite a finite permittivity of these. This would occur when part of the amniotic boundary is permeable to all species except the one under consideration and when there is bulk flow across the other part, permeable to all species. The special convection-diffusion effect treated in chapter II, section 2, is then likely to operate.

In conclusion it may be said that data on amniotic fluid composition indicate that driving forces for net transboundary transport do exist for several components. The lack of knowledge of the phase boundary properties prevents us, however, from predicting the flows that are likely to occur due to such driving forces. Also, it can be concluded that there is much evidence that the fetal urine contributes to the formation of the amniotic fluid but that its quantitative role remains undefined by this data.

2. Clinical observations

It is a well known fact that hydramnion is likely to occur when there is interference with the swallowing mechanisms of the fetus (oesophageal or duodenal atresia or strictures, encephalic malformations). This has been considered as evidence for amniotic fluid absorption in the fetal digestive (and respiratory) tract. Also, it is well known that lanugo hairs and epidermal cells may be found in the fetal stomach and intestines at postmortem examinations. PIASCEK (1947), performing estimations of the content of lanugo hairs in fetal intestine and amniotic fluid, calculated the amount of amniotic fluid swallowed by the fetus to be about 1–2 litres *in toto*, the major part of the fluid being absorbed after the 8th month of gestation. KRAUS (1951) reported a case of bleeding into the amniotic sac where the child must have swallowed a large amount of fluid at least 300 ml during the 30 hours preceding the delivery.

In cases of oligohydramnion, malformations within the urinary tract have often been found. The incidence is high enough to speculate whether fetal micturition normally plays a role in the formation of amniotic fluid. Although, as was pointed out in the preceding section, most workers agree that the fetus urinates, there is divergent opinion as to the magnitude of the diuresis and its possible role in amniotic fluid formation.

BENIRSCHKE and MCKAY (1953) have presented evidence that at least in some anencephalic children, the excess of amniotic fluid may be due to excess urine formation caused by a lack of antidiuretic hormone.

3. In vivo experiments

Experimental studies performed *in vivo* on humans and animals on the exchange of amniotic fluid have revealed that a great many components, physiological and foreign, can and do enter the compartment when driving forces are present. Some of the more important findings are discussed below.

a. Exchange and circulation of unphysiological substances.

A number of dyestuffs have been found to be able to pass from the amniotic sac into the fetus and the mother. WISLOCKI (1920) injected trypan blue into the amniotic cavity in guinea pigs and cats and concluded that this substance is absorbed during the latter part of the pregnancy by way of the gastrointestinal tract, the respiratory tract and by way of diffusion through the amniotic membrane. Phenolsulphonephthalein, cochineal and saffron were shown by BOUCEK and RENTON (1932) to be absorbed from the amniotic sac of white rats. Congo Red slowly disappears from the amniotic cavity and finds its way into the maternal circulation (DIECKMANN and DAVIS 1933). ALBANO (1934) followed the time course of the concentration of phenolsulphonephthalein injected into amniotic sacs of humans near term and found a disappearance halftime of about 7 hours. DE SNOO (1937) found that methylene blue and saccharin were excreted in the urine of women who had been given the dye in the amniotic sac.

Quinine, (FRANZ 1917, JOACHIMOVITS 1929) and barbituric acid (PLOMAN and PERSSON, personal communication) pass readily from the mother to the amniotic fluid.

Penicillin was found to be present in the amniotic fluid following intramuscular and intravenous administration of the sodium salt of penicillin to the mother (WOLTZ and ZINTEL 1945). Also FRIZEN and MEUREN (1953) found penicillin in amniotic fluid after administration to the mother and concluded, from kinetic considerations, that its appearance was at least partly due to fetal urinary secretion.

Chloral hydrate and various metabolites of that compound were found to enter the amniotic fluid in rabbits (BERNSTINE and MEYER 1953). Furthermore, it was found that during the later stage of the pregnancy, urochloralic acid, which is the form in which chloral hydrate is excreted by the kidneys, was the predominant compound.

Conclusive evidence as to the circulation of amniotic fluid in humans at different stages of pregnancy has been presented using the method of injecting radiopaque substances into the amniotic sac (EHRHARDT 1937 and 1939, REIFFERSCHEID and

SCHMIEMANN 1939, DAVIS and POTTER 1946 and ROSA 1951). These workers have established that the fetus continuously or intermittently (cp. DE SNOO 1937) swallows amniotic fluid and furthermore, that the fluid thus ingested is absorbed in the digestive tract. The rate at which swallowing occurs is not defined by these investigations, but quite large amounts, of the order of several ml per hour, seem probable from the findings. Circulation and absorption of amniotic fluid in the lungs are also established by these investigations, as the radiopaque substances are concentrated in the lungs. Again, however, quantitative data are lacking.

Repeated intravenous injections of the radiopaque substance Umbradil never gave roentgenologically significant concentrations in the amniotic fluid or in the fetus (ROSA 1951).

ROSA (1949 and 1951) investigated the distribution kinetics of inulin in humans after injection into the amniotic sac and intravenously into the mother. Trans-amniotic and transplacental transport were found to be slow. The disappearance out of the amniotic cavity followed approximately a single exponential function with a half-time of about 27 hours. The disappearance data were used for a calculation of the amount of amniotic fluid swallowed per unit time by the fetus in such a way that if B is the amount swallowed during a certain time, G is the volume of amniotic fluid and c° and c^t are the concentrations of inulin in the fluid at the two different times, the relation

$$B = G \ln (c^\circ / c^t) \tag{32}$$

should hold true. Using this formulation, ROSA found that about 500 ml was ingested per 24 hours. However, implicit in the above formulation is the rather improbable assumption that the transport of inulin across the digestive (and respiratory) mucosa occurs exclusively by bulk flow. Most probably, the figure arrived at is too large.

b. Exchange and circulation of physiological substances.

The problem of the rate of transfer of various, normally occurring, molecular and ionic components between the amniotic fluid and the maternal compartments was taken up in

1948 by FLEXNER and co-workers when isotope technique became available (VOSBURGH, FLEXNER, COWIE, HELLMAN, PROCTOR and WILDE 1948). The following principle was used. After intravenous injection of a solution containing the tagged species (Na^{24} and D_2O), a sample of amniotic fluid was withdrawn through the wall of the intact uterus in women at various times of gestation. The concentration of the tagged species was determined in the amniotic fluid and in the maternal blood, and a rapid transfer of the labelled sodium and water could be demonstrated. Later on, COX and CHALMERS (1953) confirmed the observations on sodium transfer. The problem was re-investigated by PLENTL and co-workers (PLENTL and HUTCHINSON 1953, NESLEN, HUNTER and PLENTL 1954 and HUTCHINSON, HUNTER, NESLEN and PLENTL 1955), who rightly pointed out that the theoretical treatment performed by the previous group was open to criticism. By introducing a catheter into the amniotic sac in women at term and making possible collection of amniotic fluid for about 10 hours, PLENTL and his associates followed the time course of the concentrations of injected (either into the amniotic sac or into the maternal blood) labelled sodium, potassium and water in the blood and in the amniotic fluid. The results seemed to show that the kinetics of exchange between maternal body compartments and the amniotic fluid was compatible with the assumption of two approximately equal and opposite fluxes of the species between two compartments each internally homogeneous. (Later, PLENTL (1955) suggested from preliminary data that the exchange kinetics was more in agreement with the assumption of a catenary three compartment system with exchange between the end compartments, the fetus being introduced as a third compartment). The exchange rates for the species were calculated and it was found that water was transferred at a rate of about 26 moles per hour, while sodium and potassium were transferred at rates of about 12 and 0.7 meq per hour respectively.

Some critical comments on the isotope experiments. As the relations between the self-diffusion flows and the net flows remain undefined, data obtained from such experiments can-

not be used to define the route or the mechanism of exchange. In terms of formation and resorption flow (and anatomical position) of the phase boundaries across which transfer takes place) the data cannot give any clue, a point which seems to have been overlooked by most authors. With regard to the magnitude of the exchange flows, some critical remarks may be made. The calculated exchange flows will measure the true amniotic fluid phase boundary resistance to exchange only if uniform mixing is assumed on both sides of the boundary. The assumption of uniform mixing in the amniotic compartment may perhaps be valid, but it probably fails in the maternal compartment. Concentrations of the species on the maternal side were measured in venous blood, far from the actual phase boundary and the concentration gradients so obtained are larger, may be considerably larger, than those present across the "true" boundary. Therefore, the exchange rates found should represent minimum values.

GARBY (1955 c) in a preliminary report suggested that part of the isotopic flows from mother to amniotic fluid was due to self-diffusion across the amniotic membrane. Quite recently, CHINARD and co-workers (PAUL, ENNS, REYNOLDS and CHINARD 1956) have shown that, in rabbits, about half of the exchange of water takes place across the fetal membranes and that the other half takes place across the fetus-placenta complex. These workers also conclude that the results are incompatible with the hypothesis that the exchange rates represent real volumes of water flow in and out of the system. Finally, GRAY, NESLEN and PLENTL (1956) reported values from measurements on cord blood and amniotic fluid compatible only with the assumption that at least 25 % and probably more than 50 % of the water exchange in humans occurred via the fetus.

4. In vitro experiments

Few experiments have been carried out in order to characterize the permeability properties of the amniotic membrane to matter in solution. The only works known to the present author are the qualitative ones carried out by RUNGE and co-

workers in 1927 and 1928. A number of different dye stuffs (methylene blue, Congo red, "Patent-blau" and "Cyanol") as well as glucose were shown (RUNGE 1927, RUNGE and SCHMIDT 1928) to penetrate freshly isolated human amniotic membranes, kept at 37° C in oxygenized Tyrode's or Ringer's solution (pH and CO_2-tension not specified). Also, the same dyes were found (RUNGE, BAUR and HARTMANN 1928) to penetrate the umbilical veins and appear outside the isolated perfused cord (pH, O_2 and CO_2-tensions of the perfusion and bathing fluid not specified). RUNGE and co-workers suggested, on the basis of their findings and on theoretical considerations on the pressure relationships in the system, that a fluid flow across the vein wall, cord tissue and amniotic membrane is likely to occur and that this fluid flow contributes to the amniotic fluid exchange. This interesting hypothesis has not received experimental attention.

5. On the structure of the amniotic membrane

The general structural pattern of the amniotic membrane is well known, but there seems to be some disagreement on the details. Recently, PERSSON (personal communication; to be published) has performed a systematic study of some of the histological and histochemical features of the human amniotic membrane. The description below is mainly based on his findings. The photomicrographs in figures 7 and 8 show the normal appearance.

With the exception of the fetal boundaries, the amniotic cavity is throughout bordered by the amniotic epithelium, generally a simple cuboidal (sometimes squamous and sometimes columnar) epithelium resting on a very thin membrane which is about 0.5 μ in thickness and for which the term basement membrane may be suggested. Histochemically, the latter seems to contain lipoid material and is highly refracting in ordinary light. Immediately below this membrane, a structure, optically very homogeneous and about 10–60 μ thick, forms the major constituent of the amniotic tissue. Histochemically, this structure contains collagen and mucopoly-

saccaride material. Below this layer, a very loose connective tissue stratum, about 20 μ thick, forms the connection between the amniotic tissue and the more dense connective tissue of the chorion. When the amnion is stripped from chorion, the plane of cleavage occurs in this very loose tissue which contains tiny fluid spaces, about 5–10 μ in average diameter. Very rarely, such a fluid space is seen to pierce the homogeneous layer and reach the epithelium. There is no evidence of a blood supply in the amniotic tissue.

From detailed studies of the histological structure of the amniotic epithelium, KEIFFER (1926) claimed that these cells are active in the sense that they secrete fluid into the amniotic cavity and that amniotic fluid is largely a product of the amniotic epithelium. It is difficult to see how such a definite conclusion can be drawn from the histological appearance of the cells. The possibility should be discussed that the maintenance of the basement membrane and the subepithelial structure requires metabolic activity of the epithelial cells.

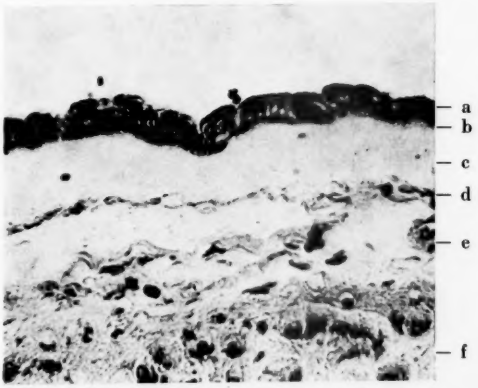

Fig. 7. Histological section of the amniotic membrane taken from a region adjacent to the placenta. a) epithelial cell layer, b) the position of the basement membrane, c) optically homogeneous connective tissue layer, d) layer of fluid spaces (plane of cleavage), e) chorionic tissue and f) decidual tissue.

Fixation: cold ethanol-methanol 1: 1. Staining: hematoxylin-eosin. x 330.

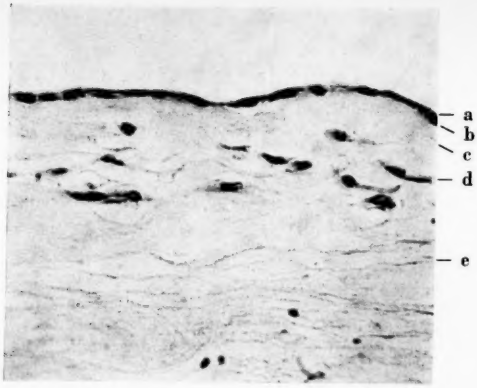

Fig. 8. Histological section from the same amniotic membrane as in fig. 7 taken from a region opposite to the placenta. The different layers are denoted as in fig. 7 and the fixation and staining procedures were the same. x 330.

Chapter IV

Experiments on the permeability properties of the isolated amniotic membrane

In the present chapter, experiments will be presented that describe how the isolated human amniotic membrane acts as resistance to flow of matter, i. e., how it prescribes the road along which the external phases tend towards equilibrium. Three closely related permeability phenomena have been investigated: 1. The diffusion and self-diffusion flow of various ions and molecules. 2. The transmembrane potential during various conditions. 3. The net volume transport resulting from "osmotic" forces.

1. Material

Amniotic membranes from normal, full time deliveries at the Department of Obstetrics and Gynecology, University Hospital, Upsala, have been used.[1] Immediately after the delivery, the membranes were stripped off from the under-lying chorionic tissue and put into glass stoppered glass flasks containing cold, freshly gassed, Krebs-Ringer's solution (composition given on p. 47). They were kept in a refrigerator at about 1° C and usually within 20 minutes they were mounted in the diffusion chambers and the experiments were begun. Usually, the specimens obtained measured about 10×10 cm. With respect to their anatomical position *in vivo*, the specimens were divided into three groups. The first group consisted of membranes taken from the vicinity of the placenta, at a hand's breadth from the placental border. The second group consisted of specimens taken from that part of the amniotic

[1] It is a pleasure to express my sincere thanks to the Head of this Department, Professor John Naeslund, for generously making material available and for his kind interest in this work.

sac which was opposite to the placenta. The last group contained membranes taken from the placental surface of the amniotic sac.

Before mounting in the chambers, the membranes were controlled by visual inspection to disclose ruptures or other irregularities. Only a few membranes were discarded, namely those where the chorionic tissue had not been properly stripped off.

After the experiments, the macroscopic appearance of the membranes was very much the same as before with the exception that the connective tissue on the chorionic side of the membranes sometimes appeared a little more gelatinous than before.

In a few cases, membranes were taken from caesarean abortions. They were treated in exactly the same way as the others.

2. The diffusion flow of various molecules and ions

Methods

The general experimental arrangement. A piece of the amniotic membrane, selected at random from the specimens obtained, was mounted between two Perspex plates measuring $70 \times 70 \times 2$ mm and each with a circular hole (diameter 25 mm) in the centre. Thin filter papers between the membrane and the plates prevented slipping. The two plates were held together with brass screws at the four corners. The unit thus obtained was mounted between two Perspex half cells, chambers I and II, each of a volume of about 35 ml and filled with Krebs-Ringer's solution. Chamber II was connected by means of rubber tubing to a large flask, serving as a reservoir and containing about 1000 ml of the Krebs-Ringer's solution. A small diaphragm pump connected in series with the system circulated the fluid between the chamber and the reservoir. The latter was placed in a water bath, the temperature of which was kept at about 40° C. The temperature in the chamber was $37°.0 \pm 0°.2$ C, the difference being due to heat loss in the rubber tubing. In the same water bath, another reservoir containing tap water was placed. By means of another diaphragm pump, the tap water circulated through a closed chamber built around the walls of chamber I. The length of the rubber tubings connecting the reservoir with the closed chamber was adjusted so that the temperature in chamber I was the same as in chamber II. In some experiments, chamber II was not connected to its reservoir, the tempera-

ture being regulated as in chamber I by means of a surrounding closed chamber.

In order to minimize the effect of convection-free layers at the membrane surfaces, the device described as follows was used. Two small rectangular Perspex plates were attached at right angles to the vibrating rod of an electro-magnetic vibrator (Pifco Massager). These plates dipped into the solutions, one on each side and close to the membrane. Previous experiments in this laboratory (Teorell, personal communication) have shown this to be quite an effective stirrer.

The Krebs-Ringer-bicarbonate solution used as a bathing fluid was made up according to COHEN (1945) and contained 118 mM NaCl, 4.72 mM KCl, 2.54 mM $CaCl_2$, 1.18 mM KH_2PO_4, 1.18 mM $MgSO_4$ and 24 mM $NaHCO_3$ per litre. In most experiments it contained in addition glucose (100 mg/100 ml). Constant gassing with 90-92 % O_2 and 8-10 % CO_2 kept the pH at about 7.3-7.4. The gas, saturated with water, was slowly bubbled through the solutions by means of glass tubings inserted to the bottom of the chambers.

The experiments were carried out as follows. About 5 minutes after mounting the membrane, the solution in chamber I was replaced by a known volume (between 20 and 25 ml) of Krebs-Ringer's solution containing the components under investigation. After another 15 minutes, during which the attainment of a steady state was assumed, aliquots were taken from this compartment at different times (usually every 15th to every 60th minute) for analysis. The experiments lasted 2-3 hours.

Calculation of the permeability coefficient. Under the conditions described, a permeability coefficient Q could be obtained by integrating the appropriate flux equation and using the condition that the species were conserved. For all species investigated, it was assumed that the differences in concentration (expressed in w/w, w/v or in counts per time and volume) between the two compartments represented the true measure of the deviation from equilibrium. The working equation (32) was thus obtained

$$Q = \frac{G_I G_{II}}{(G_I + G_{II})\,\Delta t}\, ln \frac{c^\circ_1\, G_{II}}{c^t_1\,(G_I + G_{II}) - c^\circ_1\, G_I} \qquad (32)$$

where G_I and G_{II} are the volumes of the two compartments, Δt is the time interval between two samplings with the concentrations c°_1 and c^t_1 in the compartment I. The volumes of the samples removed were always so small (less than 2 % of the compartmental volume) that they were neglected. The permeability coefficient thus calculated takes the dimension volume per unit time. The permeability coefficient Z is Q per unit area. In each experiment 3 coefficients were usually calculated corresponding to the different periods between the samplings. The mean value of these was taken as representative for the experiment (Z_m).

Determination of the concentration of the various components. The concentration of *heavy water*, present in chamber I in concentrations of

about 5 % w/w initially, was determined in the density gradient tube according to LINDERSTRÖM-LANG et al. (1938) after purification of the samples according to GARBY (1955 b). The concentration of *creatinine* (present in chamber I in concentrations of about 40 mg/100 ml initially) was determined according to the method of FOLIN and WU as described by CONSOLAZIO, JOHNSON and MAREK (1951). *Quinine*, added to chamber I to give about 5 micrograms/ml, was determined fluorometrically in sulphuric acid solution. *Radioactive sodium*, Na^{22} and Na^{24}, and *radioactive chloride*, Cl^{36}, (the sodium obtained from The Isotope Laboratory, N. V. Philips-Roxane, Amsterdam, Holland, and the chloride from the Radiochemical Centre, Amersham, England), were added to the Krebs-Ringer's solution to give about 5000 cpm/ml in the apparatus used for detection (a conventional mica end window Geiger-Müller tube and a decade pulse counter). *Radioactive siderophilin* (*transferrin*) was prepared by adding a highly purified globulin preparation (obtained by courtesy of AB Kabi, Strängnäs, Sweden, and prepared from retroplacental blood by a slight modification of the method of SURGENOR, STRONG, TAYLOR, GORDON and GIBSON (1949)) to a solution of ferric citrate containing Fe^{59} (specific activity about 200 microcuries/mg Fe, obtained as $FeCl_3$ from the Radiochemical Centre, Amersham, England). The labelled siderophilin was added to the Krebs-Ringer's solution to give about 0.05 microcuries per ml and about 0.0025 g/ml of globulin. Unlabelled siderophilin containing approximately the same amount of iron was added to the solution in chamber II. Impulse detection was made in a well type scintillation detector (Tl-activated NaI crystal) and a decade pulse counter. *Radioactive iodide and radio-iodinated albumin.* Radio-iodinated human serum albumin (The Radiochemical Centre, Amersham, England) was added to the Krebs-Ringer's solution to give about 10^4 cpm/ml (detection as for Fe^{59}) and about 0.005 mg/ml of albumin. After the experiment, both solutions were ultrafiltered to estimate the content of free iodide. An aliquot sample, kept at 37° for about 3 hours, was also subject to ultrafiltration and counting before and after the period. The membrane used for vacuumfiltration ("Kollodium-Hülzen nach Miess", Membranfilteraktiengesellschaft, Göttingen, Germany) was tested for protein leakage by running an experiment with human hemoglobin, of which less than 0.1 % appeared in the filtrate. The amount of free iodide present in the batch was quite high, about 8 %, but the rate of liberation was low, about 0.2 % per day at + 0°.5 C and negligible during 3 hours at 37° C.

Various electrochemical data were, unless otherwise stated, taken from HARNED and OWEN (1950), CONWAY (1952) and ROBINSON and STOKES (1955).

The thickness of the membranes was estimated using a micrometer caliper especially adapted for the purpose. A conventional micrometer was rotated by a weighted lever attached to the screw and at right angles to its axis. The smallest weight, with the lever in a horizontal position,

which gave a definite resistance to rotation against the membrane was used and the thickness so obtained was recorded.

The water content of the membranes was determined by measuring the loss of weight of fresh specimens, quickly dried with filter paper, after drying to constant weight at 90° C. The wet weight of the specimens was about 500 mg and weighing was performed to the nearest mg.

Estimation of the free diffusion coefficients at 37° C. The values used in the present work are shown in table 2. The value of $D^{37°}_{D_2O}$ is discussed on p. 26. The estimate refers to diffusion in pure water, but the difference between the rate of diffusion in pure water and that in a solution of the composition and ionic strength used here is presumably so small that it can be neglected for the present purposes (cf. WANG 1954). $D^{37°}_{Cl}$ and $D^{37°}_{Na}$ were calculated using the data on self-diffusion of Cl^{36} and Na^{22} obtained by NIELSEN, ADAMSON and COBBLE (1952) and MILLS (1955), the value of Cl^{36} obtained by the former group being recalculated on the basis of the remeasurement by MILLS on Na^{22}. They were corrected to 37° C with the help of values for activation energies obtained from data on limiting ionic conductance (CONWAY 1952 p. 144). The value of $D^{37°}_{Cl}$ was used for $D^{37°}_{I}$ as well, as the data on limiting ionic conductance agree within 1 %. $D^{37°}_{creat.}$ and $D^{37°}_{quin.}$ were obtained by applying the empirical relation between molecular weight and diffusion rate at 1° C found by LONGSWORTH (1952). The activation energy for diffusion of these components was obtained from data on activation energies for similar compounds found by LONGSWORTH (1954). The mean value of the activation energies of alanine and dextrose was supposed to be the best value for creatinine. For quinine, the mean value of the activation energies for dextrose and cycloheptaamylose was taken. It should be noted that owing to the approximate nature of the empirical relation between molecular weight and diffusion rate used, the error in the values of $D^{37°}_{creat.}$ and $D^{37°}_{quin.}$ may be fairly large. LONGSWORTH's data indicate that for creatinine the true value probably falls within ± 10 % of the estimated and that for quinine the value is within ± 25 % of the calculated. Errors of this magnitude may however be neglected for the present purposes. $D^{37°}_{albumin}$ was obtained by using the values at 13° and 25° C obtained by LONGSWORTH (1954).

Results

The results from measurements of the permeation of the various ionic and neutral species on some 70 amniotic specimens are summarized in *table* 1 which shows the *permeability coefficients* and their standard error in the specimens collected from various parts of the amniotic sac.

In *table* 2, all values are pooled and the coefficients of regression and their standard error of the relation between

permittivity (Z) and time are shown. The coefficients of regression were calculated with the assumption of a straight line relationship between the variates. In no case, these coefficients were significantly different from zero. In this table, the free diffusion coefficients at 37° C of the various components are also shown.

The specific restriction imposed by the membrane to the different species is shown in *tables* 3 and 4. In *table* 3, the ratios of the permeability coefficients, R_Z, obtained in membranes where these were measured simultaneously, are compared with the ratios of the free diffusion coefficients, R_D, for the particular species. In *table* 4, the differences between the ratios of the permeability coefficients and the free diffusion coefficients are shown for components not measured simultaneously.

In 3 experiments, measurements of the permeation of Cl^{36} and quinine across membranes taken at caesarean abortions were performed. The value of Z_m for Cl^{36} was 1.32×10^{-4} and for quinine 1.23×10^{-4}. The age of the fetuses was about 3 months.

In a few experiments the "spontaneous" production of creatinine and quinine of the membranes was tested by running an experiment with creatinine-free and quinine-free bathing solutions. No substances producing colour with the method used for creatinine or quinine were found.

The *thickness of the membranes*, measured on 22 specimens, varied around 0.007 cm (range 0.004–0.010 cm) with no significant differences between different parts of the amniotic sac.

The effective diffusion areas of the membranes, as calculated by comparing the free diffusion coefficients and the parameters obtained by multiplying the permeability coefficients by the membrane thickness, were found to be of the order of 1–20 % of the total membrane area.

The *water content* of the 5 specimens investigated was 90 % (range 85–96 %).

Table 1

The permeability coefficients Z_m for the different components, their standard error and the anatomical position of the specimens investigated.—(1) denotes specimens taken from the vicinity of the placenta, at a hand's breadth from the

placental border, (2) denotes specimens taken from the part opposite to the placenta and (3) specimens from the placental amniotic membrane. *n* is the number of experiments.

Component		$Z_m \times 10^4$	*n*	$\varepsilon(Z_m \times 10^4)$
D_2O	(1)	3.62	27	0.295
Cl^{36}	(1)	1.78	14	0.131
	(2)	1.49	11	0.174
	(3)	1.68	5	0.252
Na^{22}, Na^{24}	(1)	1.54	16	0.188
	(2)	1.76	7	0.236
Creatinine	(1)	0.88	13	0.145
	(3)	0.87	5	0.224
Quinine	(1)	1.28	14	0.234
	(2)	1.46	17	0.108
Fe^{59}	(1)	0.097	1	
	(3)	0.052	1	
I^{131}	(1)	1.37	1	
	(2)	2.20	1	
	(3)	2.10	1	
I^{131}-alb.	(1)	0.019	1	
	(2)	0.024	1	
	(3)	0.018	1	

Table 2

The permeability coefficients, Z_m, of the various components, their standard error, the coefficients of regression, *b*, of the permeability coefficients upon time, the standard error of the regression coefficients and the free diffusion coefficients, *D*.

Component	$Z_m \times 10^4$	*n*	$\varepsilon(Z_m \times 10^4)$	$b \times 10^4$ cm · sec^{-1}/hr	$\varepsilon(b \times 10^4)$	$D \times 10^5$
D_2O	3.62	27	0.295	— 0.20	0.20	3.03
Cl^{36}	1.66	30	0.104	— 0.04	0.08	2.58
Na^{22}, Na^{24}	1.61	23	0.144	— 0.06	0.12	1.80
Creatinine	0.88	18	0.128	— 0.02	0.03	1.13
Quinine	1.38	31	0.123	— 0.05	0.42	0.62
Fe^{59}	0.075	2				
I^{131}	1.89	3				2.58
I^{131}-albumin	0.020	3				0.091

Table 3

The ratios, R_Z, of the permeability coefficients of different pairs of components measured simultaneously, the ratios, R_D, of their respective free diffusion coefficients and the probability, P, that the differences between the two ratios are due to random factors.

Components	$R_Z \pm \varepsilon(R_Z)$	n	R_D	P
$\frac{D_2O}{Cl^{36}}$	1.72 ± 0.114	14	1.17	< 0.001
$\frac{Cl^{36}}{Creat.}$	3.30 ± 0.534	11	2.28	> 0.05
$\frac{D_2O}{Creat.}$	5.88 ± 0.737	13	2.68	< 0.001
$\frac{Na^{22}}{Quin.}$	1.51 ± 0.198	21	2.90	< 0.001
$\frac{Cl^{36}}{Quin.}$	0.964 ± 0.108	10	4.16	< 0.001
$\frac{I^{131}}{I^{131}\text{-album.}}$	94.5	3	28.4	

Table 4

The difference between the ratios of the permeability coefficients and the free diffusion coefficients for certain pairs of components not measured simultaneously, the standard error of the difference and the probability, P, that the difference is due to random factors.

Components i, j.	$\frac{Z_{mi}}{D_i} - \frac{Z_{mj}}{D_j}$	$\varepsilon\left(\frac{Z_{mi}}{D_i} - \frac{Z_{mj}}{D_j}\right)$	P
Na^{22} (24), Cl^{36}	2.51	0.896	< 0.01
Quinine, creatin.	14.5	2.28	< 0.001
Quinine, D_2O	10.3	2.21	< 0.001
D_2O, albumin	9.75		
Creatin. albumin	5.59		

Discussion

The experiments described in this section provide data as to the behaviour of the isolated amniotic membrane towards diffusion and self-diffusion of a number of molecular and ionic species. Besides the information on transport dynamics, the studies also contribute to the knowledge of the functional organisation and ultra-structure of the membrane.

For all the species investigated the resistance to transmembrane migration was found to be finite and roughly the same in the different regions of the amniotic sac. The latter finding is in accordance with the results obtained by PERSSON (personal communication, to be published) that the variations of the histological picture of the membrane do not seem to be correlated to the site of the specimens.

Considering the high water content and the relatively small effective areas for molecular and ionic penetration (1–20 %), the conclusion may be drawn that the membrane is functionally non-homogeneous and that there exist, in series, two or more layers of different permittivities. This conclusion is in accordance with the histological evidences discussed previously and with the findings of PERSSON (personal communication, to be published) that the resistance of the membranes to liquid flow decreases enormously when the epithelium is damaged. Most likely then, the homogeneous and watery connective tissue layer underlying the epithelium offers, as most connective tissues do, little resistance to diffusion, and the epithelial layer with its basement membrane constitutes the rate controlling structure. In view of its thinness, the effective penetration area should be very small.

The membrane also interacts specifically with the various components during permeation. As is evident from tables 3 and 4, the membrane acts as a sieve towards the larger molecules, in particular, albumin is considerably restricted compared with creatinine and the latter passes much more slowly compared with water than can be accounted for by differences in free diffusion. The latter finding probably reflects the presence of a majority of very small channels of permeation, the radii of equivalent cylindrical channels being of the order of 5–10 Å,

the differential restriction being accomplished by steric hindrance in the sense used by PAPPENHEIMER and co-workers. Such an interpretation is also in accordance with the results obtained on sodium and chloride permeation in comparison with water and creatinine. However, a pure steric hindrance cannot explain all the experimental data. The fact that the chloride ions are hampered compared with the sodium ions is not compatible with such a simple model and should be discussed on the basis of the "fixed charge" theory of Teorell–Meyer–Sievers. (See section 3, chapter IV.) Accordingly, the matrix of the diffusion rate controlling structure of the membrane should contain a surplus of negatively charged groups, possibly originating from proteins. Such a view is quite in accordance with the notion of small channels as expressed above. The behaviour of the highly lipoid soluble quinine molecule is striking. Compared with this molecule, even the small water molecule is restricted. The finding strongly suggests that the structures controlling permeation contain, in addition, lipoid material.

At pH 7.3-7.4, the lipoid-soluble quinine base ($pK_{b'} = 5.7$ at 20° C according to CHRISTOPHERS 1937) is present in only about 10 % of the total quinine concentration. If the positive quinine ion is less permeable than the base, the value of the permeability coefficient of the latter component should be larger than that calculated on the basis of the total quinine.

The finite permittivity to albumin molecules suggests that quite a number of channels are large enough to let such molecules through. The dimensions of such channels must be at least about 100 Å in diameter.

The exchange rate of Fe^{59} across the membrane is very low, only about 10 % of the value of creatinine which should represent a particle of about the same size. Whether the transport of the labelled iron involves exchange of ions in the free state or merely reflects globulin penetration remains unknown, but the finding that albumin molecules can pass the membrane suggests that a finite permittivity to globulins also exists. This suggestion is in accordance with the findings of BRAMBELL and co-workers (see BRAMBELL 1954), who found that, in rabbits, the amniotic membrane is permeable to immune globulins.

It may be interesting to compare the results obtained on the amniotic membrane with results of similar investigations on other biological membranes. Unfortunately, such data are scanty and they refer to systems not immediately comparable with each other. In frog skins, the available data indicate a much tighter molecular sieve, water exchange being some 100 times larger than urea exchange and some 50 times larger than chloride exchange (GARBY and LINDERHOLM 1953 and 1954). There is probably little selectivity to ions in the sense of Teorell–Meyer–Sievers (LINDERHOLM 1952). Frog gastric mucosa also performs a sieve-like function (DURBIN, FRANK and SOLOMON 1956), indeed quite similar to that found in amniotic membranes. Blood capillaries have been investigated in a similar way (PAPPENHEIMER and co-workers 1951, PAPPENHEIMER 1953, WALLENIUS 1954 and GROTTE 1956) and they, also, perform as molecular sieves. However, the channels available for permeation are generally larger than in amniotic membranes, frog skins and frog gastric mucosa. Still, the capillary walls generally show differentiation between water and smaller solutes. RENKIN (1953) showed that lipoid soluble molecules penetrate the capillary wall much more rapidly than could be accounted for by a pure steric model. Red blood cell membranes restrict small ions compared to water, facilitate the passage of lipoid soluble molecules and show a considerable ionic selectivity, anions being preferred.

Although of course rather arbitrary on the molecular scale, a differentiation between steric, electrical and chemical interaction between the membrane molecules and solution molecules seems convenient. All three phenomena are at work in the amniotic membrane as well as in other biological membranes.

The data obtained here should also provide the proportionality factors to be used in the relation between the diffusion flows of the various components and their respective driving forces, the latter being expressed in terms of concentration (activity) of the components in the surrounding phases. These problems, as well as the implications of the present findings on the experiments performed *in vivo* will be postponed until the general discussion.

3. The transmembrane potential

A knowledge of the transmembrane potential in systems such as the present is valuable for two main reasons. Firstly, the existence of "active" ion transport (cf. USSING 1949 and TEORELL 1949) may be detected with the help of such measurements. Secondly, potential data during various imposed conditions may be used to get information about the existence of fixed charges in the membrane in the sense of Teorell–Meyer–Sievers.

In the present section, data from transmembrane potential measurements will be reported, and the information that may be obtained from such data will be discussed.

Methods

Amniotic specimens were collected as described previously. In all, measurements were performed on eight membranes. They were mounted between the two Perspex plates and the two Perspex half chambers as described above. Rubber gaskets between the plates and the chamber halves prevented metallic conduction between the two compartments. These were connected to large reservoirs (about 1 litre) by means of rubber tubing, and the fluids were circulated by means of small diaphragm pumps. The reservoirs were placed in a constant temperature bath which kept the temperature in the chambers at 37°.0 ± 0°.2 C. Stirring and gassing was performed as described previously and the Krebs-Ringer's solution used was the same as before. When necessary, it was diluted by distilled water. Calomel electrodes (Beckman, fiber type) dipped into the two compartments on either side of, and close to the membrane. The potential difference was recorded on a mirror galvanometer (Multiflex) coupled to the electrodes by way of a cathode follower. A built-in, fixed voltage standard of 15 mV gave a deflection on the galvanometer of 29 scale divisions corresponding to 27 mm. It was frequently used for calibration between the measurements. The voltage standard was checked against a Weston normal cell. The potential measurements usually lasted for some 30 minutes during which time the electrodes constantly dipped into the solution. During this period it was generally possible to obtain steady potentials for three different systems: 1. Ringer's solution on both sides, 2. 1/10 Ringer on one side and Ringer on the other, 3. 1/100 Ringer on one side and 1/10 Ringer on the other. In some experiments a fourth system was used: 1/1000 Ringer against 1/100 Ringer. At the end of each measurement or series of measurements, solutions of equal composition, usually undiluted Krebs-Ringer, were placed in the two chambers and a hole made in the membrane. The potential measured under this con-

dition was taken as the "asymmetry" potential of the circuit. Any measured potential was corrected for this "asymmetry" potential which was always very near 3 mV.

Results

The electrical potential difference between the two solutions bathing the membrane was recorded in a number of experiments. Most of these are shown in table 5. With Krebs-Ringer's solution on both sides, the potential was always within 1.0 mV from zero. In one experiment, the temperature was changed from 37° C to about 21° C during the measurement. No significant effect on the potential was noted. With the system 1/100 Ringer : 1/1000 Ringer, the potential was always more positive than with the other systems.

Table 5

The potential difference (mV) across the membranes for various external fluid compositions. KR is Krebs-Ringer's solution. The sign of the potential is with respect to the solution to the right.

Experiment	KR| |KR	KR| |0.1 KR	0.1 KR| |0.01 KR
232	0.0	— 6.3	— 3.8
252	— 0.1	— 4.5	+ 4.0
262	0.0	— 5.7	— 3.6
282	0.0	— 6.9	— 4.9
73	+ 0.2	— 8.0	+ 1.2
123	— 0.5	— 6.5	— 2.0

The membrane in experiment 252 was treated with 20 % formaldehyde for 30 minutes immediately after the measurements. This treatment changed the membrane matrix so that at KR\| \|0.1 KR the potential was — 1.4 mV and at 0.1 KR\| \|0.01 KR it was + 6.8 mV, the sign of the potential being taken with respect to the dilute solution.

Discussion

As no potential difference could be recorded across the membrane when the two bathing solutions were identical it may be

concluded that the possibility that the membranes transport ions "actively" is small. It is improbable that a mechanism works upon two ions of opposite charge with such a result that both ions get the same transference.

The potential difference at finite concentration differences is difficult to evaluate (cf. eq. (12)). Any deviation from the values predicted by Planck's "constrained diffusion" boundary potential would certainly indicate membrane interference, but as the transference of uncharged species remains unknown, membrane interaction in terms of fixed charges cannot be quantitatively evaluated. However, with a reservation that the conditions are relatively unphysiological a qualitative estimation can be made. The fact that the potential varies in a consistent way with decreasing total concentration strongly suggests that fixed charges are operative, being "swamped out" at higher concentrations. This is in agreement with the findings of the tracer experiments described in the previous section.

Other possible sources of the change in the potential difference include changes in activity coefficients of the solutes and changes in the "extra" liquid junctions of the calomel electrodes. The effect of changes in activity coefficients is small, less than 0.5 mV in the case of NaCl. The assumption that the liquid junction potentials of the KCl junctions of the calomel electrodes remain essentially constant when different solute concentrations are used can be justified from the findings of TEORELL (1936). Working with calomel electrodes with KCl bridges very similar to those used in the present work, this author found essentially negligible changes in the liquid junction potentials when different solute concentrations were used.

Although the data do not allow a quantitative estimation of the concentration of fixed charges, they indicate very strongly that this is too small to explain the action of the membrane on the sodium and chloride transfer found in the previous section. Assuming that the ratio of the mobilities of these species in the membrane is the same as in water, it can be calculated according to the formulations of TEORELL (1953) that the concentration of the fixed charges must be of the order of 100 mM/l in order to explain the relative values of the permeability coefficients of sodium and chloride. Such a high concentration of fixed charges is incompatible with the small change in transmembrane potential when different total concentrations are

used (cp. TEORELL 1953). In order to explain the results, one might venture the hypothesis that sodium and chloride ions penetrate the rate controlling structure of the membrane partly by way of non-aqueous channels where specific binding or solubility occurs.

Ion selectivity is known to occur in many biological membranes. The relative role of fixed charges in this ion selection is not elucidated. MANECKE and OTTO-LAUPENMÜHLEN (1953) and WALLIS (1954) investigated connective tissue membranes from various sources of guinea-pigs, rats and frogs and found evidence of fixed charges of small concentrations, about 1–5 mM/l, the influence of flow of uncharged species being however neglected in their calculations. In general, the results presented in this paper, including those regarding the effect of the treatment with formaldehyde, are in accordance with those of MANECKE and OTTO-LAUPENMÜHLEN and WALLIS.

4. The net liquid flow

In this section, an attempt will be made to evaluate the rate and direction of the net liquid flow across the amniotic membrane during *in vivo* conditions. This procedure requires the use of the relations developed in chapter I, section 3, some of the data obtained in section 3 of this chapter, a knowledge of the composition and pressure of the two phases surrounding the membrane *in vivo* and further experiments on isolated membranes. It has not been possible to define exactly the rate of flow during *in vivo* conditions, but the results obtained have been assumed to be sufficiently accurate to allow some important conclusions.

Generally, the *rate of liquid flow* across a membrane is given by the *conductance to flow* × the appropriate *driving force*. Obviously, the conductance may depend both on the membrane structure and the magnitude of the driving forces. The latter will depend on the membrane structure if they are given in terms of the intensive properties of the surrounding phases. Little is known of these relations in biological systems. For

the purpose of the present considerations it is convenient to distinguish sharply between membranes where the migrating species pass without mutual interaction, and membranes where they do interact (cf. USSING 1952, GARBY 1955 a). *The first case* has been treated rather extensively by LAIDLER and SHULER (1949 a and b), who give the net flow in the form

$$B = \frac{k_1 A \cdot c_t \tilde{V}_1^2}{d \cdot RT} (RT \Delta c_2 - \Delta p) - \frac{k_2 A \cdot c_t \tilde{V}_1 \tilde{V}_2}{d \cdot RT} \cdot RT \Delta c_2 \qquad (33)$$

where k_1 and k_2 are the permeation coefficients, with respect to the membrane, of the solvent and solute, respectively, (k_1 not being measured by tracer permeation); c_t is the total concentration of all species and the subscripts of c and V refer to solvent and solute, respectively. Δc_2 and Δp is the difference in concentration and pressure on both sides of the membrane. The solution is ideal.

If *the second case* is considered, the net liquid flow will be described by eq. (24) (cf. p. 22).

The data on the permeation coefficients obtained in the previous section indicate that the amniotic membrane occupies an intermediate position with respect to the two models. The finite permeation of a molecule such as albumin makes it highly probable that small molecules, such as water, pass the membrane with mutual interaction. Therefore, part of the matter transfer should be accomplished by viscous flow in which case eq. (24) would be valid. On the other hand, the relation between the permeation coefficients of water and creatinine indicate that a number of channels exist in the membrane where it is difficult to imagine a viscous flow mechanism.

The net liquid flow in the first case. It is possible then to estimate the flow of liquid across the membrane *in vivo* by considering the model where no interaction during passage is permitted. The net flow of liquid is given by

$$B = \sum_i A \cdot Z_i V_i (a_i^{\mathrm{I}} - a_i^{\mathrm{II}}) \qquad (34)$$

where the superscripts denote the two surrounding phases and

the summation is over all permeating species. Electrical and thermal forces as well as pressure forces are assumed to be negligibly small. The total area of the membrane *in vivo* can be estimated approximately as follows. The total volume of the amniotic sac content was determined by ETTERICH (1948) to be 4400 ml (3500–5500 ml). The area of a prolate spheroid of the volume 4400 ml and with the axis 34 cm and 15.7 cm is 1420 cm^2 and with the axis 28 cm and 17.4 cm it is 1341 cm^2. If volumes of 3500 ml, axes 30 cm and 15 cm, and 5500 ml, axes 36 cm and 17.8 cm, are assumed, the areas are 1202 cm^2 and 1638 cm^2, respectively. From these considerations it is evident that a value of 1400 ± 200 cm^2 is a good average value of the area of the amniotic membrane *in vivo*. If eq. (34) is applied to the following species: water, sodium chloride, urea, creatinine and glucose, it can be shown that B becomes an almost negligible quantity, of the order of 20–30 ml per 24 hours. Other permeating species do not contribute significantly to this quantity. In the calculation of B, the water activities have been calculated from the freezing point depressions (cf. p. 35), the difference in activity of sodium chloride, urea, creatinine and glucose has been assumed to be 10 mM/l, 3.8 mM/l, 0.24 mM/l and 4.44 mM/l, respectively (cf. FRIEDBERG 1955 and HANON and co-workers 1955) and the permeation coefficients for urea and glucose have been taken to be $2.0 \times \times 10^{-4}$ and 0.5×10^{-4} cm sec^{-1}, respectively. In spite of the relative uncertainty in the calculation of B, it can be safely concluded that, if the mechanism of net fluid permeation is that predicted by the first model, *the rate of flow of liquid is very small, of the order of 20–30 ml per 24 hours and that the flow is in the direction from the amniotic cavity into the extracellular fluid of the mother.*

The net liquid flow in the second case. It remains to estimate the direction and rate of flow in the case when the membrane permits also viscous flow. It is however evident that, on the basis of the present knowledge, such a calculation cannot be made. Firstly, the laws governing viscous flow in channels of the dimensions of the present system are not known, secondly, the pore size distribution, essential for the applica-

tion of eq. (24), is not defined and thirdly, the relative restriction compared to water of all species present is unknown. Finally, the importance of uniform mixing of the solute and solvent in the phases surrounding the membrane should be remembered. If such mixing cannot occur, the energy available in the form of steady concentration differences between the two phases will be partly lost by diffusion processes near the membrane surface where unstirred layers exist (cf. KUHN 1951 and the discussion on p. 65).

It is possible to make quite reasonable estimations of all the unknown variables except two, the possible occurrence of a few, but very large pores and the possible loss of energy in unstirred layers, and it is precisely these possibilities that may make such calculations quite erroneous. If, for example, it is assumed that no channels available for flow are more than 1000 Å in radius, eq. (24) together with the Hagen-Poiseuille law and the data obtained on the permeation coefficients may be used in various reasonable ways and combinations to give a net flow of about 50–200 ml per 24 hours in the direction from the amniotic sac into the maternal system. If, however, it is not possible to rule out the existance of pores or "leaks" with radii of as much as 10^4 Å, the presence of such pores, even it they constitute not more than 0.1 % of the total area available for flow, will lower the resistance to hydraulic flow to such an extent that hydrostatic pressure differences of only about 4 cm of water will cause a net flow of about 1000 ml per 24 hours in the direction of the hydrostatic pressure drop. Obviously, it is not possible to rule out either the occurrence of such pores or such hydrostatic pressure differences on the basis of the available data.

The experiments to be described were made in order to establish whether or not the amniotic membrane contains pores of this order of magnitude and to estimate, if possible, at least the maximum rate of flow across the membrane.

Methods

Amniotic specimens were collected as described previously. They were mounted between two Perspex plates, $2 \times 60 \times 120$ mm and $4 \times 60 \times 120$ mm, the thinner one containing a circular hole (diameter 43.6 mm) in its

middle. The other plate had a slightly concave excavation corresponding in site and form to the hole in the other plate. The concave part of this plate contained numerous small holes (diameter about 1.5 mm) very close to each other. The unit so obtained was mounted between two Perspex chambers I and II, forming in principle, a system like that shown in figure 5. Chamber I, total volume about 140 cm^3, was however open and contained Krebs-Ringer's solution (about 65 ml) constantly renewed from a reservoir in a temperature bath which kept the temperature of the solution at 37°.0 ± 0°.2 C. Chamber II (total volume about 78 cm^3) and III were kept at the same temperature by means of closed chambers built around the walls of the chambers. The capillary tube connecting chamber II and III had a bore of 1.2 mm and contained during operation a drop of liquefied phenol. Chamber III was mounted on a cathetometer to permit controlled vertical movement of this chamber. The cathetometer scale permitted accurate readings of 0.01 mm, but smaller movement could be made and approximately estimated by the position of the operating screw.

The sensitivity and accuracy of the measuring system was estimated in the following way. A Perspex plate was mounted between the chambers I and II and the system was filled with Krebs-Ringer's solution. When the volumes of the solutions in chambers II and III had equilibrated and the temperature had become constant, a drop of liquefied phenol was introduced into the capillary. By moving vertically chamber III, it was possible to determine the relative inertia of the system. When chamber III was raised or lowered about 0.01 mm, the drop always moved regardless of how it had reached its "equilibrium" position. This operation corresponded to an increase (or decrease) of about 28 mm^3 in chamber III and a passage of about 7 mm^3 of fluid along the capillary, since the ratio of the open areas of chamber II to chamber III was 0.308. It was possible, by adjusting the height of chamber III with movements of less than 0.01 mm, to place the drop in such an "equilibrium" position that a calculated flow of less than 7 mm^3 along the capillary caused it to move. When stationary temperatures in the solutions had been obtained, the movement of the drop during 60 minutes was always less than 4 mm, corresponding to a flow of less than 4.5 mm^3 per hour. When the "equilibrium" position had been found, a constant rate of fluid flow was delivered into chamber II by using the apparatus for continuous injection described by Öbrink (1948). When a flow of 27 mm^3 per hour was delivered, the drop always started to move within about 5-10 minutes. The rate of movement of the drop increased from small values during the next 5-10 minutes and generally showed some overshooting so that when the injection stopped, it continued to move for some 5-10 minutes. However, after an hour of constant addition of fluid to chamber II, the movement of the drop corresponded to within 80-120 % of the expected value. Thus, a net flow of 27 mm^3 per hour to chamber II was readily detected. The relatively small inertia of the system was obtained only when the capillary was meticulous-

ly cleaned and when the drop of liquefied phenol was small, about 3 mm in length.

Results

Four membranes from different parts of the amniotic sac (positions 1, 1, 2 and 3 respectively) were investigated. The membranes were mounted so that the epithelial side faced the solution in chamber II. The compartments were not stirred, but the solution in chamber I was bubbled through with gas as described earlier. The hydrostatic pressure difference between chambers I and II was adjusted to about 3 cm of Krebs-Ringer's solution so that the pressure was higher in chamber II and the membrane rested on the perforated plate. The membrane area exposed was 15 cm^2. In this situation, with Krebs-Ringer's solution on both sides, the movement of the drop in the capillary was in all cases less than 1.5 mm (1.1, 0.5, 0.8 and 1.5 mm) during 30 minutes corresponding to a net decrement of volume in chamber II of less than 4.4 mm^3 per hour. When a flow of 27 mm^3 per hour was added to chamber II with the apparatus described above and the pressure difference was 3 cm of Krebs-Ringer's solution, the drop moved significantly during one hour, corresponding quite nearly to the flow added (81, 94, 83 and 91 % respectively).

When Krebs-Ringer's solution containing no glucose and 10 mM/l less of NaCl was used in chamber II, there was a significant movement during two hours of the drop in the capillary, corresponding to a net decrement in volume of chamber II of 15, 19, 11 and 21 mm^3 per hour respectively.

Discussion

Considering the various errors in the method, it can be concluded that driving forces of about the magnitude expected *in vivo* produce significant net liquid flow across the amniotic membrane. The direction of the liquid flow is from the amniotic cavity into the maternal system if the hydrostatic pressure is the same, within some 5 cm of water, on both sides of the membrane. The rate of flow cannot be exactly evaluated on the basis of the present experiments, but the following con-

clusions can be drawn as to its approximate magnitude. A hydrostatic pressure difference between the two surrounding media of 3 cm of water or less is very unlikely to produce a flow of more than 5–10 mm^3 per hour and 15 cm^2 membrane area. This corresponds to not more than 11–22 ml per 24 hours totally. The apparent "osmotic" pressure in the present experiments, corresponding to 5.5 mM/l of glucose and 10 mM/l of NaCl and to $0^\circ.05$ C difference in freezing point depression, is certainly far from that calculated according to the van't Hoff relation (= 0.68 atm). Using eq. (23) and a value of $0.1 - 0.5 \times \times 10^{-4}$ cm sec^{-1} for glucose permeation, it is found to be about 0.26–0.30 atm. This value is most probably a maximum value for the true "osmotic" pressure difference across the membrane and it is presumably not far from the true value. The contribution of other species to the pressure gradient, such as creatinine, urea and uric acid, for which concentration gradients exist, is small, of the order of 10^{-5} to 10^{-6} atm.

The rate of "osmotic" flow in the present experiments was between 11 and 21 mm^3 per hour. This flow rate may be overestimated by some 2–4 mm^3 per hour (cf. above) but it is not likely to be underestimated. Considering the possibility that some 4 mm^3 was not detected (cf. above), *a probable daily flow of 7–65 ml per total amnion is obtained.* This value is in agreement with the value calculated theoretically on the basis that material transport occurs in accordance with the model of LAIDLER and SHULER, i. e. sorption and desorption at the membrane boundaries and individual migration within the membrane. The presence of water-filled pores permitting viscous flow cannot be excluded. However, the "osmotic" pressure drop in these channels is relatively small and probably does not contribute appreciably to the net liquid flow.

This is due to the relatively large leakage of the membrane to solute molecules, but the possibility should also be discussed that insufficient mixing in the phases surrounding the membrane may lower the effective driving pressure. KUHN (1951) performed a thorough analysis of the effect of liquid flow on the solute concentration profile in the unstirred layers at the membrane. He deduced relations between the van't Hoff pressure and the effective driving pressure in terms of the liquid flow rate across the membrane, the dimensions of the unstirred layers and the

magnitude of the diffusion coefficient of the solute. Consequently, the parameters entering his relations (eq:s (28) and (28 b) p. 214) are similar to those used in the convention-diffusion equations of chapter III, section 1 and 2, and chapter VI, section 2, and the calculations made in the latter section can be directly used to evaluate the effect of the unstirred layers in the present case. The condition for the effect of unstirred layers to be small is that the quotient $v \cdot l/D \ll 1$ where v is the linear velocity of flow of liquid at the membrane surface, l is the extension of the unstirred layer into the bulk phase and D is the diffusion coefficient of the solute in the solution. This condition can be shown to be fulfilled in the present case even when the value of l is one centimeter.

Hydrostatic pressure differences of the order of magnitude likely to occur in the present system produce small flows, a fact that seems to exclude the presence of any large number of "leaks" of the dimensions 10^4 Å or more in the membrane. In fact, the flow characteristic of the membrane is such that a fraction of the total area available for water permeation of less than 2×10^{-5} is permitted for channels with a radius of 10^4 Å and less than 2×10^{-3} for channels with a radius of 10^3 Å.

Chapter V

General discussion

In this chapter, the evidence obtained by earlier workers and that presented here will be considered in assessing the role of the amniotic membrane in the exchange of amniotic fluid. In the system as a whole, several important processes are poorly defined as previously pointed out. Therefore, a certain lack of cohesion between the various contributions makes it difficult to obtain a unified picture of the dynamics of the system.

1. Some remarks concerning the validity of the in vitro experiments as applied to in vivo conditions

Although the experimental conditions closely imitate those *in vivo*, it is by no means clear that the data obtained can be used for calculations of *in vivo* events. The medium surrounding the membrane differs from that of amniotic fluid and extracellular fluid in many respects. The Krebs-Ringer's solution contains no proteins, amino acids and other organic metabolites such as creatine, creatinine, lactic acid etc. It probably contains more bicarbonate than amniotic fluid and its hydrogen ion concentration is slightly less (cf. MANNHERZ 1949). Although it is not clear how these differences would change the conditions to any large extent, this possibility cannot be excluded. The fact that the permittivity of the amniotic membranes to various components did not change appreciably (cf. table 2) during the time of the experiments, indicates that the membranes remained relatively unaffected by the experimental conditions.

The present findings, as well as the findings of PERSSON, strongly indicates that the rate controlling structure for material transport across the membrane is situated in the epi-

thelial layer and/or its basal membrane. As these layers are nourished by diffusion, they should be unaffected by the isolation from the underlying chorionic tissue. Also, the kinetics of penetration should, from this point of view, be unaffected by the isolation and the values obtained should therefore reflect those *in vivo*.

2. The net flows across the amniotic membrane

The data on the resistance to diffusion and mass flow of the amniotic membrane obtained in this work can be used to calculate the net flows of the various species across the membrane during *in vivo* conditions. However, the characteristics of the system do not allow exact estimations. In order to perform such calculations, i. e. the use of the equations (6)–(9), it is necessary to know 1. the values of the driving forces across the membrane *in vivo*, i. e., the concentration gradients, the electrical potential gradient, the hydrostatic pressure gradient and the temperature gradient, 2. the values of all L_{ik} where $i \neq k$, 3. the value of v and its variation between different areas of penetration and 4. the value of the *in vivo* area of the membrane.

The values of the concentration gradients of various species across the membrane can be evaluated from the measured concentrations in the amniotic fluid and in the extracellular maternal fluid. In most cases sufficiently accurate data are available. The electrical potential across the membrane *in vivo* is most probably so small that it can be neglected for the present purposes (cf. chapter IV, section 3) and the same also applies to the hydrostatic pressure and temperature gradients. The variation along the x-axis of the concentration gradient during conditions of no electrical, hydrostatic or temperature gradients depend on the magnitude of v and can be evaluated.

The coefficients L_{ik} with $i \neq k$ as defined here are codiffusion coefficients, i. e., they describe the influence of any migrating species on the others' movements with respect to the mass centre. There exist no measurements of the relative magnitude of these coefficients. However, in dilute solutions, the

cross coefficients between the dilute components must be small compared to the other L_{ik}:s. DUNLOP and GOSTING (1955) and FUJITA and GOSTING (1956) estimated particle interaction in three component systems during diffusion. Although their results cannot be directly interpreted in terms of the L_{ik}:s defined here, the measurements indicate that these must be so small that they can be neglected for the present purposes.

The magnitude of the area of the amniotic membrane *in vivo* has been discussed on p. 61.

The net flows can then be evaluated by the application of eq. (10), integrated with respect to the x-axis. However, the relative heteroporosity of the membrane makes the direct use of this relation questionable and must be discussed. The following modification of the integrated form of eq. (10) is supposed to be a better description of the process

$$\sum_{k=1}^{n} J_{ik}^{m} = \sum_{k=1}^{n} A_k \frac{D'_{ik}}{d}\left(a_i^{\mathrm{I}} - a_i^{\mathrm{II}}\, exp\, \frac{v_k d}{D'_{ik}}\right) \frac{v_k d/D'_{ik}}{exp\,(v_k d/D'_{ik}) - 1} \qquad (35)^*$$

where the summation is over all n different pore classes. When $v_k d/D'_k \to 0$, Fick's law is obtained. The application of eq. (35) is difficult because little is known of the relations between the pore dimensions and flow resistance on one hand and between the pore dimensions and the value of $\Delta\pi$ on the other hand. It should be noted however that the situation may be simplified to allow approximations if the condition $\Delta p = 0$ is considered. In this case, it can be shown that the variation of v between the different pore classes, where $\Delta\pi$ is at all effective, is relatively small. This is due to the fact that while $\Delta\pi$ increases with decreasing pore dimensions, the conductance for flow decreases with decreasing pore dimensions. In the present case, it can be shown that the effect of plausible values of v on the diffusion kinetics is small and negligible for the present purposes. Let us assume, for the sake of argument, that the amniotic membrane is homoporous. The value of 50 ml

*Eq. (35) is a modified form of a special case of a general formula for a single electrolyte convection-diffusion case derived by TEORELL (unpublished, personal communication).

per 24 hours totally of the net liquid flow (0.579×10^{-3} ml sec^{-1}) gives for $v \cdot d$, which is $B/\left(\frac{A}{d}\right)_w$ where $\left(\frac{A}{d}\right)_w$ is equal to $A \cdot Z_w/D_w$, a value of 3.5×10^{-8} cm^2 sec^{-1}. The meaning of $\left(\frac{A}{d}\right)_w$ is this: it is the area per unit path length of the membrane that is available for water to diffuse as in "free" solution. The coefficient D' is not less than $0.3 \times D$ for small solutes like NaCl, KCl and urea and not less than $0.2 \times D$ for species like creatinine and glucose. The value of $v \cdot d/D'$ is then 0.0057 and 0.015 and the value of $\exp v \cdot d/D'$ is 1.0057 and 1.015, respectively. Inserting in eq. (35), for all k-values equal, it is found that the deviation from the case where $v = 0$ is quite negligible.

A similar conclusion with respect to the linear velocity of flow in capillary membranes was also reached by GROTTE (1956), who based his calculations on the data of PAPPENHEIMER (1953) and on calculations of the distorsion of the concentration profile (according to TEORELL, personal communication, unpublished) within the membrane (assumed homoporous).

Most probably, a heteroporous system with the pore distribution characteristics of the amniotic membrane and with the condition $\Delta p \ll \Delta \pi$, will not deviate appreciably from the above calculations.

Recently, HODGKIN and KEYNES (1955) have suggested a very special membrane model in order to account for deviations from theory in the case of potassium movements across nerve membranes. In their model, the species under consideration is supposed to move in a single file along narrow channels or along chains of sites which stretch through the membrane. Whatever the detailed molecular model may be, the situation requires that changes of sites are permitted only when the whole row of particles move. The important point is that in such a situation, independant movement of the particles in the membrane is prohibited and the information gained by measurements of isotopic exchange rates is difficult or impossible to interprete. While it is difficult to exclude, in principle, the possibility of such a mechanism of migration, it is even more difficult to imagine a structure where the intermolecular forces are such as to prevent interchange of neighbours while a movement in a row is permitted. Incidentally, it should be remarked that the case discussed by HODGKIN and KEYNES is covered in the general macroscopic theory as outlined in chapter II. Here, interdependancy of movement is taken care of by strictly defining diffusion relative to the movement of the centre of gravity. The findings of HODGKIN and KEYNES may have an explana-

tion in the movement of water across the membrane, in which case the possibility of an interchange of neighbours in the membrane remains.

It remains to discuss the choice of the values of the concentrations of the various species in the extracellular fluid bathing the chorionic side of the membrane. Although plasma values are usually a very good measure of the extracellular fluid composition for small molecules and ions (corrected for the Donnan effect), this is not necessarily so. If the rate of transport from the extracellular fluid spaces between the amnion and the chorion to the plasma is not very large compared with the rate of transport across the amniotic membrane itself, gradients of concentrations in the extracellular fluid spaces will exist, directed towards the plasma compartment. The concentration differences between plasma and amniotic fluid will then be larger than those between amniotic fluid and the fluid in the extracellular spaces in contact with the amniotic membrane. Accordingly, the use of plasma concentration values in the present case could lead to an overestimation of the driving forces for flow. The deviations from the true values are determined by the relative magnitudes of the transport rates discussed above. Because of the differences in diffusion path lengths and the morphological features of the structures, it is most probable that the rate of transport from the extracellular fluid to plasma is much larger than that across the amniotic membrane. The error from assuming plasma and extracellular concentrations to be the same should thus be small.

It can then be concluded that the net flows of the various species, except water, can be calculated by the expression

$$J_i^m = A \cdot Z_i (a_i^{\mathrm{I}} - a_i^{\mathrm{II}}) \qquad (36)$$

and that the errors introduced by this simplified formula are small enough to make the estimations important practically.

The net flow of water can be taken to be the same as the net liquid flow estimated previously and is of the order of 0.3–2.7 ml/hr in the direction from the amniotic sac into the maternal compartment. *The net flow of NaCl* is 0.3 gm/hr, assuming a Δc_i of 8 mM/l (cf. HANON and coworkers 1955 and HUTCHINSON and coworkers 1955) and an activity coefficient

of 0.75. The direction of flow is from the maternal compartment into the amniotic cavity. *The net flow of KCl* is 0.05 gm/hr in the same direction, assuming the same permeation and activity coefficients as were taken for NaCl and a Δc_i of 1 mM/l (cf. HANON and coworkers 1955 and HUTCHINSON and coworkers 1955). For *urea* and *creatinine,* values of 0.11 and 0.012 gm/hr out of the amniotic cavity are found if Δc_i is 11 and 2.7 mg/100 ml, respectively, and a permeation coefficient of 2.0×10^{-4} cm sec^{-1} for urea is assumed. A value of $0.1 - 0.5 \times \times 10^{-4}$ cm sec^{-1} for *glucose* permeation gives a value of the flow of glucose of 0.04–0.19 gm/hr into the amniotic cavity ($\Delta c_i = 75$ mg/100 ml). The net flow of *albumin* is difficult to evaluate because of the lack of knowledge of Δc_i, but it probably never exceeds 0.05 gm/hr in either direction.

Net flows of other species can be estimated at least in the right order of magnitude if their concentrations in the amniotic fluid and in the plasma are known, because the uncertainty in the assumed value of Z_i should generally be less than a factor of 10.

In terms of *amniotic fluid net turn-over* across the amniotic boundary, the following approximate figures are calculated: water 0.1 %/hr, NaCl 3 %/hr, KCl 15 %/hr, urea 30 %/hr, creatinine 30 %/hr and glucose 30 %/hr.

The values obtained here, as well as the values of the total flows (cf. section 3), refer to the situation at the time of delivery. During earlier stages of gestation, other values for the magnitude of the net flows and the total flows must be expected. Generally, if the permeation coefficients remain essentially constant, which is, in fact, indicated by the findings of the membranes from abortions (cf. p. 50), all flows are likely to show a decrease with decreasing gestation time. This is due to the fact that all components, with the possible exception of glucose, show a tendency to equalize in concentration with decreasing gestation time and also that the area of the membrane decreases. Thus, between the 4th and 5th month of gestation, there are no significant concentration gradients of urea, creatinine and uric acid (FRIEDBERG 1955) and the difference in freezing point depression is only about 0°.03 C.

3. The total flows across the amniotic membrane

In agreement with the simplifications outlined in the previous section, the total (unidirectional) flows across the amniotic membrane can be calculated according to

$$J_{io}^{m} = A \cdot Z_i \cdot a_{ii} \tag{37}$$

where the subscripts denote the flow out of the cavity and the activity in the amniotic fluid. A corresponding relation is found for the flow into the sac. The total flows into and out of the cavity are very nearly the same for water, sodium and chloride, the values being 100 moles/hr, 76 millimoles/hr and 63 millimoles/hr. The value for potassium is of the order of 3 millimoles/hr if a permeation coefficient of the same order as that of sodium is assumed.

The values of the total flows are about 4–6 times larger than those found by HUTCHINSON et al. (1955) on the total exchange *in vivo*. They found, for water a value of 26 moles/hr (range 15–42), for sodium a value of 12 millimoles/hr (range 7–15) and for potassium a value of 0.7 millimoles/hr (range 0.4–1.4). Because of the probability that mixing in maternal plasma and extracellular fluid is non-uniform (cf. the discussion on p. 41), the results obtained *in vivo* are not directly comparable with the results of the present work. The results indicate, however, that, if the condition of the membrane *in vitro* is not changed, *part of the flows calculated by the use of isotopes in vivo are due to self-diffusion and have little to do with formation-reabsorption flows*. The suggestion of non-uniform mixing *in vivo* is further illustrated by the good agreement of the present results and the *in vivo* experiments on the exchange of larger molecules which may be expected to be less dependant on good mixing. Thus, the data obtained by NESLEN, HUTCHINSON, HALLET and PLENTL (1954) on the disappearance of I^{131}-tagged albumin from amniotic fluid show a half-time of about 73 hours while the corresponding half-time calculated on the basis of the present data is 69 hours.

Data on the disappearance of inulin and phenolsulphonephthalein from the amniotic sac of humans *in vivo* are also

of interest in this connection. A half-time value of about 27 and 6 hours respectively can be calculated from the data of ROSA (1949) and ALBANO (1934). If the exchange of these components across the fetal boundaries is relatively small, the half-time values can be interpreted in terms of Z for phenolsulphonephthalein and inulin. The values are found to be 0.23×10^{-4} and 0.05×10^{-4} cm sec^{-1} respectively, which are in good agreement with the values expected from the average molecular size of these components (cf. table 2).

It is possible, finally, to make a comparison between the present results of water exchange across the isolated membrane and those presented by PAUL and coworkers (1956) on the water exchange across amniotic membranes of rabbits *in vivo* with the fetus crushed. From their data, a value of Z of 1.80×10^{-4} cm sec^{-1} for water can be calculated. The mixing in these experiments was probably better than in the experiments in humans because the amniotic sac was massaged during the experiment. The agreement with the present *in vitro* experiments is good.

4. Physiological implications and concluding remarks

The amniotic membrane is one of the boundaries interposed between the maternal extracellular fluid and the amniotic fluid. Its general properties as a barrier to matter transport have been discussed above. The physiological significance of this barrier is not clear. The fluid phase situated between the membrane and the fetus at the time of delivery is quite similar in intensive properties to that of "ordinary" extracellular fluid and is probably identical with it during earlier stages of gestation. During the later months of gestation, the composition of the fluid changes slightly but significantly. These changes are in complete agreement with the hypothesis that fetal urine enters the cavity. The fluid increases in amount, becomes diluted and accumulates nitrogenous excretion products. The rate of urine formation during fetal life remains unknown.

If the amniotic phase boundary were completely impermeable, the removal of such excretion products and the excess

volume should be wholly dependant upon other possible sites of exchange (the fetus and the cord). The present investigation indicates that waste products (originating from urine and/or other possible sites) can be removed from the amniotic fluid by way of a simple diffusion process across the amniotic membrane.

The progressive dilution of the amniotic fluid, by the addition of hypotonic fluid, increases considerably the water activity inside the sac and provides a potential force for liquid flow out of the cavity across the amniotic membrane. However, its permeability properties are such as to prevent liquid flow to any greater extent. Therefore, if fetal diuresis exceeds the capacity of this outflow mechanism, other sites of outflow must exist. It is reasonable to assume that the digestive and respiratory tract mucosa are the sites of additional fluid flow out of the amniotic cavity.

As a result of the dilution process, some components, for instance Na^+, K^+ and Cl^-, cross the amniotic membrane in the direction of the amniotic fluid. Glucose probably traverses the membrane in the same direction during the whole gestation period.

The membrane also permits small amounts of proteins to pass. The significance of this phenomenon is not understood, but it is possible that this route is of importance in the transfer of high molecular weight components from mother to fetus.

The possible role of the amniotic membrane in the production of excess amniotic fluid should be discussed. Evidently, a change to complete impermeability would cause fluid accumulation if a compensatory outflow across the fetal boundaries fails. The possibility that this situation may be of importance in acute or chronic hydramnion seems to have been ruled out by the findings of HUTCHINSON and coworkers (1955) that the water exchange remains essentially unaltered in such cases. Another explanation of a diminished or reversed flow would be the situation where the channels of permeation have become so large that "osmotic" forces are negligible or zero. This situation may be brought about without changing appreciably the total area available for diffusion and may thus escape

detection by a measurement of water exchange rates. In such a situation, the rate of flow would be completely determined by the hydrostatic pressure difference on both sides of the membrane. However, it is difficult to imagine that the hydrostatic pressure between the amniotic membrane and the chorion is larger than that in the amniotic cavity during conditions of hydramnion. The possibility of a change in the driving forces for flow by a change in amniotic fluid composition is perhaps not likely to account for cases of acute hydramnion because of the extremely large flow rates required, but it is quite possible that this mechanism may work in cases of chronic hydramnion. It is now known that the total solute concentration in amniotic fluid is larger than normal in cases of hydramnion (HANON and coworkers 1955 and HUTCHINSON and coworkers 1955). It should be pointed out however that little is known of the possible changes of the flow at other sites of liquid exchange. Such changes may well be large enough to produce fluid accumulation of the rate required to explain the conditions in hydramnion.

Summary

The purpose of the present investigations is stated in the introduction. It has been to study, from a general physiological point of view, the permeability properties of the amniotic membrane and to examine the results in terms of amniotic fluid exchange in humans.

In *chapter I*, section 1, some basic concepts and definitions concerning liquid exchange in biological systems are given. Section 2 contains an outline of the theories of irreversible transport of matter with special reference to transfer across membranes. In section 3, parts of the theory are considered in more detail, and a contribution to the treatment of ordinary osmosis is given. The relations derived and discussed are used in the following.

Chapter II contains a presentation of some experiments carried out on artificial membranes. They deal with the influence of net mass transfer on the kinetics of transmembrane transport. For the porous membranes investigated, the theoretical relations are shown to be valid.

In *chapter III*, the results and conclusions regarding amniotic fluid circulation and exchange obtained by earlier investigations are discussed. It is concluded that little is known of the site, the mechanism and the rate of exchange of the components of the amniotic fluid.

Chapter IV presents experimental studies on the permeability properties of the isolated amniotic membrane. The material is described in section 1. Section 2 presents experiments on the diffusion resistance of the membrane to the following species (the label used for tagging is shown in parenthesis): water (D_2O), sodium (Na^{22}, Na^{24}), chloride (Cl^{36}), iodide (I^{131}), creatinine, quinine, iron (Fe^{59}) and albumin (I^{131}-labelled). The data on the rate of transmembrane diffusion are used to evaluate the functional ultrastructure of the membrane. In section 3, the electrical potential difference across the membrane,

measured during various external total concentrations, is discussed and interpreted in terms of "active" transport and membrane ultrastructure. Section 4 deals with experiments performed in order to evaluate the net liquid flow across the membrane during various conditions. Also here, the data are used to characterize the functional ultrastructure of the membrane.

The following conclusions are drawn on the basis of the data obtained in this chapter. The amniotic membrane is permeable to all species investigated including albumin. It shows steric restriction to permeation indicating that most channels available for penetration are of molecular dimensions, i. e. less than about 100 Å. More specifically, a large part of the area available for penetration probably consists of channels with dimensions of the order of 5–10–20 Å. Thus, the difference between the permeation rates of water, creatinine and albumin are much larger than can be accounted for by differences in the mobility in water. Ions, such as Na^+ and Cl^- and I^- are also restricted in their passage compared to water. The lipoid-soluble quinine base shows facilitated transfer in comparison with lipoid-insoluble molecules of about the same size, a fact that indicates the presence of lipoids in the rate controlling structure of the membrane. This structure also seems to contain a low concentration of fixed negative charges. The relative rates of penetration of Na^+ and Cl^- indicate that these components are transferred at least partly by way of specific binding or solubility in the membrane. The structure of the membrane as a whole is such that water is preferred compared to the majority of the normally occurring solutes so that a finite "osmotic" flow results when one of the surrounding phases is diluted. The relative number of very large "leaks", with dimensions of 10^3–10^4 Å, is small and negligible in calculations of the resistance to liquid flow.

Chapter V contains the calculations, from the theoretical and experimental evidence, of the net flows and total unidirectional flows of the various components across the membrane during *in vivo* conditions. The physiological significance of the membrane is discussed and it is concluded that, although

the net liquid flow across it is small, of the order of 0.3–3.0 ml/hr in the direction out of the amniotic cavity, the net transfer of some species is not negligible and may have a physiological significance. The approximate values for the net flow rate of the various components are: NaCl and KCl: 0.3 and 0.05 gm/hr respectively into the amniotic compartment; urea and creatinine: 0.1 and 0.01 gm/hr out of the amniotic cavity; glucose: 0.05–0.2 gm/hr into the sac and albumin: less than 0.05 gm/hr in either direction.

The large isotopic flows obtained during *in vivo* experiments by earlier investigators are interpreted as consisting at least partly of self-diffusion.

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